EFFECTS OF HYDROPEAKING AND REFUGE CONFIGURATIONS ON THE BEHAVIOUR OF CYPRINIDS IN EXPERIMENTAL FLUME CONDITIONS

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
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Dedico esta tese aos meus pais e ao meu irmão.

Título: Efeitos do *hydropeaking* e de diferentes configurações de abrigos no comportamento de ciprinídeos ibéricos num canal experimental.

Resumo

O regime de escoamento é essencial para manter a integridade ecológica dos ecossistemas fluviais. As rápidas alterações de caudal a jusante das centrais hidroelétricas (i.e. *hydropeaking*) decorrentes das variações do consumo de energia induzem alterações significativas do regime de escoamento. Estas, em conjunto com as alterações morfológicas e de qualidade da água daí decorrentes, afetam as comunidades piscícolas. Estabelecer uma relação de causa-efeito entre a variabilidade do escoamento e a resposta da ictiofauna é desafiante, tornando-se difícil propor medidas de mitigação adequadas. A primeira parte desta investigação consistiu numa revisão bibliográfica para encontrar informação sobre aquela relação. Diversos estudos demonstraram que a variabilidade do escoamento pode representar um potencial *stress* para a ictiofauna. No entanto, encontra-se por determinar se as alterações fisiológicas correspondem a uma resposta provisória ou permanente. Na segunda parte, os efeitos do *hydropeaking* e da presença de abrigos foram avaliados num canal experimental para o *L. bocagei*. Testaram-se diversas situações de *hydropeaking*, variando o caudal máximo, a frequência de variação e a respetiva duração. Testaram-se abrigos com diferentes geometrias: defletores (alternados ou unilaterais), e estruturas triangulares (pirâmides sólidas ou estruturas em V). Determinaram-se as concentrações de glucose e lactato, as frequências de uso dos abrigos e a atividade no canal. Caracterizaram-se o escoamento e as interações fluído-corpo através de um velocímetro acústico por efeito de Doppler (ADV), e de uma sonda de secção hidrodinâmica dotada de uma linha de sensores de pressão (LLP), procurando representar a linha lateral dos peixes. O comportamento foi diversificado e nem sempre proporcional à severidade do *hydropeaking*. Os defletores e as estruturas em V proporcionaram zonas de velocidade reduzida. No entanto, a complexidade do escoamento resultante reduziu a capacidade dos peixes para os encontrar. Determinaram-se limites de velocidade que asseguram o repouso do *L. bocagei*, estabeleceram-se relações entre as respostas e as alterações hidrodinâmicas e propuseram-se recomendações para medidas morfológicas e operacionais para mitigação do *hydropeaking*.

Palavras-chave

Hydropeaking, L. bocagei, abrigos, comportamento, linha lateral.

Title: Effects of hydropeaking and refuge configurations on the behaviour of cyprinids in experimental flume conditions.

Abstract

Flow regime regulates the ecological integrity of river ecosystems, shaping the structure and function of fish communities. The discharge fluctuations in hydropower plants in response to peak electricity demand (i.e. hydropeaking) result in rapid flow changes in tailwaters. The continued hydropower operations produced morphological, hydraulic and water quality alterations, affecting downstream fish. Fish responses to hydropeaking range from organism to life-cycle event changes. It is challenging to establish a cause-effect relationship between flow variability and a fish response, and to propose adequate mitigation measures. In the first part of this research, a literature review was conducted to find evidence for that relationship. The review showed that flow variability can represent a stressor for fish. However, it remained unclear if the responses were maladaptive. In the second part, the effects of hydropeaking and refuges were assessed for *L. bocagei* in an indoor flume. A multidisciplinary approach was adopted, where fish responses were combined with a hydraulic characterization. Peak events were tested by manipulating magnitude, peak frequency and duration. The refuges were lateral (meandering and one-sided deflectors) and instream (triangular pyramids and v-shaped) structures, tested along three experimental campaigns. Glucose and lactate (secondary responses), and movement behaviour (whole-animal responses) were assessed. The flow field and fluid-body interactions were characterized by using acoustic Doppler velocimetry and an artificial lateral line probe respectively. The movement patterns of *L. bocagei* were diverse and not always proportional to the severity of the flow event. Lateral deflectors and v-shaped structures provided low velocity areas. However, the created flow complexity represented an additional constraint for fish, reducing their ability to find them. Flow thresholds that represented the resting state of *L. bocagei* were identified, and specific movement patterns were related with hydrodynamic changes. Practical recommendations for operational schemes and for the implementation of mitigation measures to hydropeaking were proposed.

Keywords

Hydropeaking, *L. bocagei*, refuges, physiology, movement behaviour.

Título: Efeitos do *hydropeaking* e de diferentes configurações de abrigos no comportamento de ciprinídeos ibéricos num canal experimental.

Resumo Alargado

A variabilidade natural do regime de escoamento regula os processos físicos e biológicos que ocorrem no ecossistema fluvial, modelando a estrutura e função das suas comunidades. Devido à força evolutiva deste regime, as espécies que habitam estes ecossistemas desenvolveram características adaptativas para responder a tal variabilidade. Numa perspetiva temporal, a variabilidade intra- e interanual do regime de escoamento exerceu uma forte pressão evolutiva, por exemplo no que se refere a características morfológicas e reprodutivas. Por sua vez, a variabilidade intra-anual exerce uma pressão ecológica, atuando em atividades como a procura de refúgio, busca de alimento ou fuga de predadores. Considerando o papel regulador do regime de escoamento, qualquer perturbação natural ou antropogénica poderá afetar a estrutura das comunidades piscícolas.

A rápida expansão da população humana tem ocasionado uma utilização progressivamente mais intensa dos recursos hídricos. O progresso da agricultura através da irrigação, a melhoria da qualidade de vida através do desenvolvimento de estruturas de abastecimento e tratamento de água, o fornecimento de hidroeletricidade e o controlo de cheias são alguns dos benefícios daí retirados. No entanto, de tal utilização resultou a fragmentação dos ecossistemas fluviais e a redução da sua biodiversidade. A expansão global da produção de energia hidroelétrica é tida como uma das causas mais preocupantes para a sustentabilidade destes ecossistemas e para a resiliência das suas comunidades. A frequente variabilidade dos caudais turbinados nas centrais hidroelétricas (i.e. *hydropeaking*) para corresponder à variação da procura da energia elétrica ao longo do dia, induz alterações significativas do regime de escoamento a jusante. Estas, em conjunto com as alterações morfológicas e de qualidade da água daí decorrentes, afetam as comunidades piscícolas. As repostas da ictiofauna ao *hydropeaking* podem-se manifestar em diferentes níveis de organização biológica, como alterações fisiológicas e metabólicas, ou em alterações no recrutamento, crescimento ou reprodução. Estes efeitos foram extensivamente estudados em alguns grupos de ictiofauna (ex. salmonídeos). No entanto, não foi possível determinar uma relação causa-efeito entre a variabilidade do regime de escoamento e uma resposta mensurável, dificultando a conceção de medidas de mitigação adequadas. Em rios afetados por *hydropeaking*, registaram-se alterações laterais e longitudinais no movimento dos peixes, de maior ou menor amplitude. Outros estudos não constataram qualquer alteração do movimento, ou não foram bem-sucedidos na explicação de determinados padrões do movimento. Na impossibilidade de observar as preferências de habitat dos peixes, estes estudos atribuíram a variabilidade do movimento à potencial disponibilidade de refúgios. Embora os efeitos do *hydropeaking* tenham sido estudados, não foram propostas medidas de mitigação morfológicas para minimizar as suas consequências.

As instalações experimentais permitem controlar variáveis externas, manipular os parâmetros hidráulicos, e observar padrões de comportamento numa escala mais refinada. Desta forma, estudos realizados em instalações experimentais propuseram diferentes tipos de substrato, cobertura, estruturas semelhantes a defletores, ou zonas de descanso, a partir de respostas fisiológicas e da avaliação do comportamento e da performance de salmonídeos. Por outro lado, os efeitos do *hydropeaking* e a conceção de medidas de mitigação para ciprinídeos, encontram-se pouco estudados. No entanto, os ciprinídeos constituem a maior parte das comunidades de peixes da Península Ibérica, o número de endemismos é elevado e devido ao efeito biogeográfico e ao clima maioritariamente mediterrânico, as comunidade de ciprinídeos dos rios Ibéricos apresentam características adaptativas únicas.

Esta investigação dividiu-se em duas partes. Na primeira enquadraram-se os seguintes objetivos principais: (i) encontrar evidência científica para a relação causa-efeito entre a variabilidade no regime de escoamento e a existência de uma reposta mensurável dos peixes; (ii) fornecer conhecimento à comunidade científica para a seleção de respostas fisiológicas a utilizar como biomarcadores em condições de regime de escoamento natural e modificado. Na segunda, os objetivos principais foram: (i) avaliar os efeitos de eventos de caudal-base e *hydropeaking* na fisiologia e comportamento do *L. bocagei* num canal experimental equipado com estruturas artificiais tidas como abrigos; (ii) conceptualizar geometrias alternativas para a mitigação do *hydropeaking*; (iii) avaliar a atividade do *L. bocagei* na presença e ausência de abrigos; (iv) identificar os eventos de *hydropeaking* com o maior e o menor efeito de acordo com as respostas quantificadas; (v) encontrar limites críticos e favoráveis de velocidade e de condições hidrodinâmicas de acordo com as repostas quantificadas e, (vi) propor recomendações para a implementação de medidas morfológicas de mitigação do *hydropeaking*.

Os estudos analisados para a revisão bibliográfica (capítulo 2) mostraram que a variabilidade natural ou antropogénica do escoamento pode alterar a homeostasia do organismo, manifestada por ajustes fisiológicos. Os estudos analisados demonstraram que existe uma relação entre aquela variabilidade e uma reposta provisória do organismo. No entanto, está por determinar se as alterações fisiológicas correspondem a uma reposta provisória ou permanente. No entanto, estes estudos sugerem que o dispêndio energético associado a estas alterações fisiológicas poderá repercutir-se por exemplo, no crescimento, reprodução e sobrevivência das espécies piscícolas.

A segunda parte desta investigação consistiu numa bateria de experiências realizadas durante três campanhas experimentais (capítulos 3 e 4). Usando uma abordagem multidisciplinar, esta componente da investigação foi realizada num canal experimental localizado no Laboratório de Hidráulica e Ambiente do departamento de Engenharia Civil do Instituto Superior Técnico. Para avaliar o efeito do *hydropeaking*, testaram-se diversos eventos variando o caudal máximo, a frequência da variação, a duração do caudal máximo e a duração total do evento. Os caudais testados (7, 20, 40 e 60 $l.s^{-1}$) foram integrados nos diferentes eventos consoante as hipóteses formuladas. Para simular condições de

escoamento-base, os peixes foram sujeitos a um evento contínuo a 7l.s^{-1} com uma duração correspondente a cada evento-pico testado. Uma vez que o canal experimental apresenta um fundo amovível, testaram-se diferentes geometrias para avaliar o seu potencial sucesso como refúgio: defletores (dispostos alternadamente, capítulo 3.1; ou dispostos unilateralmente, capítulo 3.2), e estruturas triangulares (pirâmides sólidas ou estruturas em V, capítulo 4). O escoamento e as interações fluído-corpo foram caraterizados através de um velocímetro acústico por efeito de Doppler (ADV) (capítulo 3) e de uma sonda de secção hidrodinâmica dotada de uma linha de sensores de pressão (LLP), procurando representar a linha lateral dos peixes (capítulo 4) respetivamente. Os resultados obtidos através do ADV foram calibrados num modelo numérico tridimensional (FLOW-3D®). Este permitiu simular outras configurações e caraterizar o escoamento consoante o evento e a estrutura testados. Através da LLP mediram-se as diferenças de pressão entre o corpo da sonda e o fluido. Determinaram-se as concentrações de glicose e de lactato como respostas fisiológicas às condições hidráulicas criadas pela combinação evento-estrutura. Para avaliar os padrões de movimento dos peixes, determinaram-se as frequências individuais e em grupo do uso das estruturas, os *sprints* e os arrastamentos.

Os modelos FLOW-3D® evidenciaram áreas no interior dos defletores com velocidades <0.1 cm.s^{-1} para todos os caudais testados. No entanto, foram observadas velocidades de 0.72 cm.s^{-1} e de 0.68 cm.s^{-1} para o caudal mais elevado na presença de deflectores alternados e unilaterais, respetivamente. As maiores diferenças de pressão foram registadas a jusante das estruturas triangulares (pirâmides sólidas e estruturas em V), onde se verificou inclusive uma maior complexidade das condições hidrodinâmicas. Mesmo que a pressão registada na área interior das estruturas em V tenha sido menor comparativamente à pressão a jusante das pirâmides sólidas, as condições hidrodinâmicas circundantes foram mais complexas.

Os resultados das experiências demonstraram que o comportamento foi diversificado e nem sempre proporcional à severidade do *hydropeaking*. Por exemplo aquando da simulação de *hydropeaking* na presença de estruturas, registaram-se ajustes fisiológicos e uma maior frequência de *sprints* individuais. Por outro lado, o comportamento de grupo foi mais marcado nos eventos-base e em situações de *hydropeaking* sem estruturas. Verificaram-se ajustes de glicose em situações de *hydropeaking* com defletores, enquanto os aumentos de lactato foram apenas verificados no evento *hydropeaking* na presença de estruturas em V. Os modelos FLOW-3D® e os contornos de pressão resultantes da LLP evidenciaram que a área interior dos defletores e das estruturas em V garante características hidráulicas (velocidade) e hidrodinâmicas (pressão frontal média, flutuações frontais médias e pressão assimétrica média) favoráveis ao *L. bocagei*. No entanto, a complexidade do escoamento resultante do *hydropeaking* e da presença do abrigo, reduziu a capacidade dos peixes para usar estas estruturas como refúgio.

Concluiu-se que os diferentes padrões de comportamento resultaram das condições hidráulicas criadas em simultâneo pelo evento e pela presença das estruturas. A abordagem multidisciplinar adotada

reforçou a interpretação das respostas dos peixes, contribuindo para que se proponham medidas mais eficientes de mitigação do *hydropeaking*. A partir destes estudos determinaram-se limites de velocidade que asseguram o repouso do *L. bocagei*, e estabeleceram-se relações entre as variáveis de pressão e as respostas fisiológicas e os padrões de movimento do *L. bocagei*. Finalmente foram propostas recomendações para a conceção de medidas morfológicas e operacionais para mitigar os efeitos do *hydropeaking* no *L. bocagei*.

Palavras-chave

Hydropeaking, *L. bocagei*, abrigos, comportamento, linha lateral.

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List of Abbreviations

3D	Three-dimensional
A	Acclimation
ADV	Acoustic Doppler Velocimetry
ANOVA	Analysis of Variance
BF	Base-flow event
C	Compartment
CA	Correspondence Analysis
CEN	European Committee for Standardization (Comité Européen de Normalisation)
CEF	Forest Research Center
CERIS	Civil Engineering for Research and Innovation for Sustainability
CN	Canal Neuromast
D	Deflector
E	Experiment
EMG	Electromyogram
FCT	Portuguese Foundation for Science and Technology (Fundação para a Ciência e para a Tecnologia)
G	Group
HP	Hydropeaking
HPI	Hypothalamo-pituitary-interrenal
I	Individual
ICNF	Institute for Nature Conservation and Forests (Instituto da Conservação da Natureza e das Florestas)
INAG	National Water Institute (Instituto da Água)
IST	Instituto Superior Técnico
LLP	Lateral line probe
NACA	National Advisory Committee for Aeronautics

ND	No deflector
ODBA	Overall dynamic body acceleration
P	Period
PMCMR	Pairwise Multiple Comparisons of Mean Rank Sums
PVC	Polyvinyl chloride
R0, R1, R2	Structures (refuges)
RpUDR	Repeated up- and down-ramping
SD	Standard deviation
SEM	Standard error of the mean
SgUDR	Single up- and down-ramping
SgUR	Single up-ramping
SN	Superficial Neuromast
StUR	Step up-ramping
T	Time
TL	Total length
TW	Total weight

List of Symbols

i	pressure sensor number, cross-wall number or scenario number
j	position number in a data array or prim number
l	position of a pair of sensors
m	total number of sensors
n	total number of data in an array
$\overline{p}_i$	mean pressure in sensor i
$\overline{p}'_i$	mean fluctuations in sensor i (Pa)
$\overline{p}_{12}$	mean front pressure (Pa)
$\overline{p}_{1-6}$	mean pressure (Pa)
$\overline{p}'_{12}$	mean front fluctuations (Pa)
$\overline{p}'_{1-6}$	mean fluctuations (Pa)
p	probability value (also used P depending on the journal criteria)
u, v, w	longitudinal, transversal and vertical instantaneous velocity components
x, y, z	longitudinal, transversal and perpendicular to the flume bottom coordinates
Q	discharge or flow rate (m3/s)
W	Wilcoxon rank-sum test
α	significance level
$\Delta\overline{p}_{12}$	mean front pressure asymmetry
$\Delta\overline{p}_{1-6}$	mean pressure asymmetry
$\Delta\overline{p}'_{12}$	mean front fluctuations asymmetry
$\Delta\overline{p}'_{1-6}$	mean fluctuations asymmetry
χ2	chi-squared test

1

Introduction

“There is a mystery about rivers that draws us to them, for they rise from hidden places and travel by routes that are not always tomorrow where they might be today. “

Olivia Laing

1.1 The natural flow regime

The physical and biological processes that occur at each spatial component of the river system (Frissell et al., 1986) and between its dimensions (Ward, 1989) affect the whole river network. This hierarchical organization of river ecosystems strengthened the ecological relevance of this research because the impact of rapid flow changes in lower levels of spatial and ecological organization, would likely scale up to higher levels of the river network.

All events of a fish life-cycle occur in the riverbed. As stream dwellers, fluvial fish depend on the physical and biological processes that occur in the riverbed to successfully complete diel activities (foraging, escaping predators, and finding refuges) and life-cycle events (reproductive migrations, spawning, growth, and recruitment). During these diel and life-cycle events there are inherent movement behaviour changes that occur along the river network and generate distinct fish assemblages (Allan and Castillo, 2007; Poff et al., 1997). Flow plays a determinant role for the success of diel activities, its natural variability is a cue for life-cycle events, and its long-term temporal character determined evolutionary traits (Bunn and Arthington, 2002; Lytle and Poff, 2004). Poff et al. (1997) discussed, for the first time, the critical role of natural flow regime to sustain the ecological integrity of the river ecosystem. The natural flow regime framework postulates that its temporal variation shapes the mosaic of the physical habitat and regulates the structure and function of riparian and aquatic communities (Bunn and Arthington, 2002; Poff et al., 1997). The primary components of flow regime are: magnitude (or flow ratio), frequency, duration, timing and rate of change (Figure 1.1). These act at different spatial and temporal scales, regulating short- and long-term river ecosystem processes (Freeman et al., 2001; Gasith and Resh, 1999; Lytle and Poff, 2004).

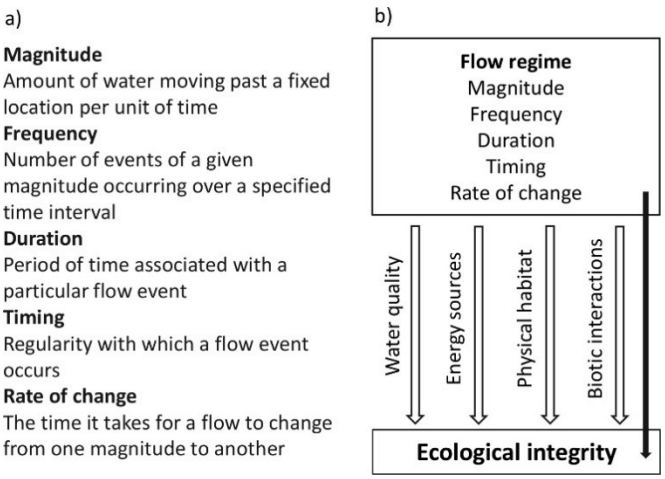

Figure 1.1 The natural flow regime. a) The five components of flow regime – magnitude, frequency duration, timing, rate of change – and, b) the cascading indirect (white arrows) and direct (black arrow) effects on the ecological integrity of the fluvial ecosystem. Adapted from Poff et al. (1997).

Natural flow disturbances are intrinsic to healthy river ecosystems (Lytle and Poff, 2004). Floods and droughts are integral for the structure of freshwater communities acting across a range of spatial and temporal scales. As a consequence, fish species have evolved adaptive traits enabling them to survive, exploit and depend on these disturbances (Lytle and Poff, 2004). The natural flow regime has an intrinsic predictability. Its natural inter-annual variability has exerted strong selection pressure at the evolutionary scale (Figure 1.2). Thus, it has defined specific traits in fish species related with biology (e.g. age at reproduction; Lytle and Poff, 2004; Resh et al., 1988), behaviour (e.g. timing of spawning migration), physiology (e.g. tolerance for thermal regime) and morphology (e.g. body shape) (Enders et al., 2009). On the other hand, the intra-annual flow variability exerts low selection pressure, and forces aquatic species to adapt, acting at the ecological scale (Lytle and Poff, 2004; Resh et al., 1988) (Figure 1.2).

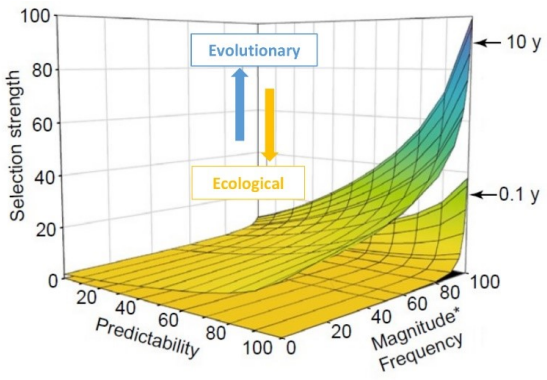

Figure 1.2 The evolutionary and ecological effects of inter- and intra-annual variability of flow regime. Adapted from Lytle and Poff (2004).

The selection strength of flow variability is particularly prominent in the river ecosystems of the Iberian Peninsula. The majority of these ecosystems are characterized by a Mediterranean climate (Ferreira et

al., 2007b; Gasith and Resh, 1999). This results in a strong intra-annual variability in the flow regime, with high flow periods during winter and limited water availability during summer (Gasith and Resh, 1999). Thus, the ichthyofauna of these highly variable systems has evolved evolutionary and ecological traits (e.g. rapid growth rates, high fecundity, or early sexual maturity and spawning) enabling them to adapt to this strong flow variability (Ferreira et al., 2007b). However, the continued modifications of the natural flow variability of Mediterranean rivers have disrupted the *river continuum* as Vannote et al. (1980) described it. In a short-term fish species are forced to adapt, however in a long-term the effects of flow modifications may become cumulative and result in biodiversity loss (Allan and Castillo, 2007).

This natural flow regime framework represented a major advance for the scientific community and for both freshwater managers and users because it emphasized the major role of flow regime for freshwater biota. By understanding the fundamental principles of natural flow variability, it would be possible to predict the ecological consequences of altering any flow component, and act towards a more naturalized river flow (Arthington et al., 2006; Poff et al., 2003). However, it is challenging to translate this knowledge into management strategies that not only comply with human demands but assure the resilience of the freshwater ecosystem (Arthington et al., 2006; Naiman et al., 2002)

1.2 Hydropower production global status and trends

From the beginning of the 20th century onwards, hydropower production has expanded as an important source of electricity. Hydropower was the largest source of renewable electricity generation in 2017, contributing to 16.4% (out of 24.5%) for the global production of renewable energy (IHA, 2018). Due to the increasing demand for an energy source that responds to societal needs for cost-effective electricity (Yüksel, 2009), hydropower is now the fastest growing end-use of energy (IHA, 2018). In addition, hydropower with reservoirs is the only renewable energy with storage possibilities (Harby and Noack, 2013; IHA, 2018). Thus, hydropower production will continue to expand (IHA, 2018; Zarfl et al., 2015). Although the positive aspects of hydroelectricity are emphasized in the International Hydropower Association report (IHA, 2018), they have to be interpreted with caution. The environmental impacts of the artificial and rapid flow fluctuations in downstream rivers (Harby and Noack, 2013) and the consequent habitat degradation and biodiversity loss (Nilsson et al., 2005) were not analysed for this report.

1.3 The particular case of hydropeaking

The effects of rapid flow fluctuations downstream of hydropower plants were firstly reviewed by Cushman (1985). In this review, the first evidence of the impacts of rapid and artificial flow changes on fish dates back to 1955, where the population of stocked rainbow trout was assessed in the tail-water below a hydroelectric dam (Parsons, 1955). Cushman (1985) firstly refers to "hydro-electric peaking" as the operational manoeuvres that occur in the hydropower plant in response to the electricity demand to control the large and rapid (within minutes) changes in the discharge by powering-on or -off the hydro-turbines, resulting in rapid flow changes in tailwaters (Cushman, 1985). Less than a decade after, Moog (1993) alarmed for the necessity to quantify the ecological impacts of peak hydropower and to propose management solutions to minimize them. By that time, the relevance that was given to the impact of the short-term flow fluctuations to river ecosystems was alarming and already considered as "one of the most serious disturbances of regulated riverine ecosystems" (Moog, 1993). If by that time the concern for this problematic was emerging, today it is an urgent matter. Presently, these rapid and artificial flow fluctuations caused by sub-daily to weekly energy demand are generally referred to as "hydropeaking".

A recent review study addressed the global trends of hydropeaking in riverine plants from 1994 to 2016 (Bejarano et al., 2018). From the 165 articles that were analysed by using the keyword "hydropeaking" as a search string, more than half were published between 2012 and 2016 (Bejarano et al., 2018). This illustrates the recent growing importance of this topic and the awareness of the scientific community to study it. The impacts of hydropeaking have been mostly studied for freshwater fauna, where fish and macroinvertebrates' studies contributed to 43 and 14% respectively (Bejarano et al., 2018). From 2010 onwards, other topics related with river hydrology, hydraulics and management solutions have been introduced (Bejarano et al., 2018). This trend is probably supported by the recent advances in computing power and landscape information (Hauer et al., 2017). These are decisive to accurately model the hydrodynamics of rivers affected by hydropeaking, and to project future scenarios accordingly (e.g. Hauer et al., 2014, 2013).

In free-flowing rivers the fluctuations in daily flows change according to: precipitation, evapotranspiration, infiltrations or snowmelt, and catchment-scale characteristics (e.g. drainage area, land use or slope) (Lundquist et al., 2002). These variations may be small in comparison to the variability at yearly time-scales. However, they affect the physical and biological processes of freshwater ecosystems (Biggs et al., 2005). Rivers affected by hydropeaking incur comparably higher dramatic flow changes in both daily and yearly timescales (Bejarano et al., 2018; Bunt et al., 1999; Zimmerman et al., 2010). The continued long-term hydropower plant operations result in strong morphological, hydraulic and water quality alterations including: bank and soil erosion and continuous shifts in the sediment transport processes (Greimel et al., 2018; Hauer et al., 2017, 2013; Moog, 1993; Schmutz et al., 2015),

changes in water level, flow velocity, water turbulence and bed shear stress (Shen and Diplas, 2010), and in water temperature (Bakken et al., 2016; Zolezzi et al., 2011), dissolved gas concentrations (Bejarano et al., 2018; Calles et al., 2007; Pulg et al., 2016) or turbidity (Bice et al., 2013; Bunt et al., 1999).

1.3.1 Characterization of hydropeaking

The discharge fluctuations occurring downstream the hydropower plant in response to the electricity demand create distinct phases of hydropeaking: 1) low base discharge, corresponding to no electricity production; 2) increasing discharge, or up-ramping, where electricity production rapidly increases; 3) continuous high peak discharge, where the demand for energy peaks; 4) decreasing discharge, or down-ramping, corresponding to the shutdown of the turbines (Bruder et al., 2016). These operations generate irregular changes in the components of flow regime, specifically: the flow ratio, rate of change, frequency, duration, and timing (Figure 1.3).

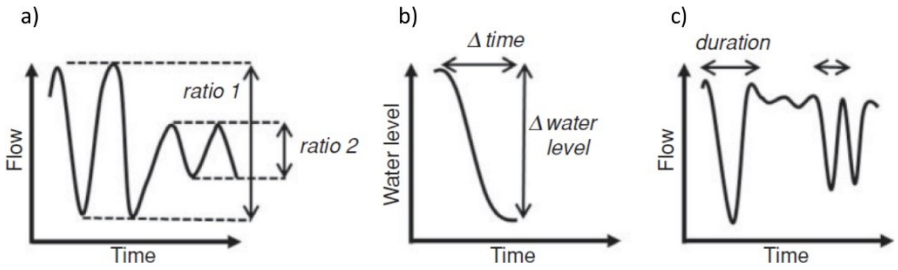

Figure 1.3 Parameters to characterize rapid flow fluctuations. a) Flow ratio: the high flow divided by the low flow; b) Rate of change: change in the water level divided by the time of that change; c) Duration: time between two peaks in the hydrograph, Frequency: how often peaking occurs (between the arrows); Timing, when peak events occur. Adapted from Harby and Noack (2013).

The characterization of these components is the basis to quantify the impacts of hydropeaking and to recommend guidelines to mitigate its consequences (Bejarano et al., 2018; Bruder et al., 2016; Harby and Noack, 2013). In hydropeaking conditions, it is frequent that one or more of these flow regime components exceed the ranges of the natural flow regime, creating the most severe impacts (Harby and Noack, 2013).

Hydropeaking results in highly variable sub-daily discharge fluctuations, with highly frequent peaks during the day. The common statistics used to define the extent of hydrologic alteration in relation to the natural flow regime, are based on daily-averaged flow records (e.g. Richter et al. 1996). This methodology fails to capture the variability that characterizes short-term flow fluctuations. Thus, to understand hydropeaking it is necessary to characterize the changes in magnitude, frequency, rate of change, duration and timing in a sub-daily scale (Meile et al., 2011). In recent years, sub-daily flow metrics have been proposed to characterize hydropeaking and to quantify its impacts, with temporal

scales ranging from 15 (Sauterleute and Charmasson, 2014), 30 (Zolezzi et al., 2011) , and 60 minutes (Alonso et al., 2017; Bevelhimer et al., 2015; Carolli et al., 2015; Meile et al., 2011).

1.3.2 Experimental approaches to study hydropeaking and its impacts

The study of hydropeaking and its impacts depends on whether the objective is to characterize the daily and sub-daily rapid flow fluctuations, to quantify the impacts of hydropeaking in the physical and biological processes of the affected river ecosystem, or to conceptualize mitigation measures. The research approaches can be generally grouped in: *in situ,* experimental flume and numerical modelling studies. These approaches can be used solely or combined.

In situ studies are usually selected to quantify the effects of hydropeaking on fish assemblages (Vehanen et al., 2005), and on specific life-cycle events (e.g. alteration of migratory cues, survival, rearing or growth). In *in situ* studies, the manipulation of flow is rarely possible (but see Krimmer et al., 2011) because it is impractical and costly for hydropower plant managers. One of the most reported disadvantages of *in situ* studies is the overlapping effects of other physical and biological variables that are difficult to isolate (Harby and Noack, 2013). In experimental flume studies (indoor or outdoor) it is possible to examine smaller scale behaviour (e.g. substrate preferences or social interactions). The main advantage of experimental flume studies is the possibility to control external variables and flow changes. However, it is commonly difficult to simulate the rapid flow fluctuations in the same order of magnitude and rate of change as in some regulated rivers due to limitations that are inherent to the facility (Harby and Noack, 2013). In addition, it is often very difficult to reproduce natural conditions of substrate, hyporheic flow, and sediment dynamics. Numerical modelling studies have been increasingly used to characterize hydropeaking and to propose morphological mitigation alternatives (Boavida et al., 2015; Hauer et al., 2017, 2014). These present valid tools to predict potential scenarios, or to develop conceptual frameworks to serve as grounds for hydropeaking studies (Bruder et al., 2016; Young et al., 2011; Zimmerman et al., 2010).

With *in situ* experiments the highest realism is achieved, in experimental flumes it is possible to control the factors under examination and to replicate the same flow conditions (Woodward et al., 2010), and with numerical models it is possible to project hypothetical flow conditions (Almeida, 2014; Hauer et al., 2014; Ribi et al., 2014). Ideally, the hydropeaking problematic should be addressed using a combination of the available approaches to strengthen the findings and to propose management recommendations (Harby and Noack, 2013).

1.3.3 Mitigation measures to hydropeaking

The mitigation measures to hydropeaking can be categorized in three types: operational, morphological and structural (Person, 2013; Person et al., 2014). Operational measures aim at adjusting

the flow regime downstream the power plant by adapting the operational scheme to be more suitable for biota; morphological measures aim at improving the river morphology towards a more naturalized state by the utilization of river engineering techniques; and structural measures aim at smoothing the impact of the magnitude and rate of change of the discharges during hydropeaking by the construction of hydraulic structures (Bruder et al., 2016; Harby and Noack, 2013; Person, 2013; Person et al., 2014).

Operational measures involve the adaptation of the production scheme to reduce the impacts for downstream biota. The most frequently and relevant suggested changes in the operation include: (i) smoothing the extreme changes in magnitude, rate of change, frequency and timing of peak (Bruder et al., 2016; Clarke et al., 2008); (ii) create a step-wise discharge increase (i.e. a pre-surge) (Bruder et al., 2016); (iii) attenuating the ramping rate because it should mimic a rapid natural flow increase (Auer et al., 2017; Clarke et al., 2008), and (iv) reducing or increasing the minimum flows during critical spawning, rearing and recruitment (Casas-Mulet et al., 2014; Clarke et al., 2008; Pragana et al., 2017). Although operational measures seem an adequate solution, it is challenging to find a trade-off between the economic outcome for the hydropower industry and the ecological benefits (Jager and Smith, 2008; Pragana et al., 2017), in addition to the technical difficulty associated with powering-on and –off the hydro-turbines (Harby and Noack, 2013).

The morphological measures involve the addition of artificial or natural structures in the river bed, or the modification of the river channel and floodplain to reduce the negative impacts of hydropeaking (Harby and Noack, 2013; Person, 2013). Enhancing river morphology adds heterogeneity which ensures refuge availability. However, it also interacts with the changes in the hydraulic conditions associated with hydropeaking (Casas-Mulet et al., 2015a; Vanzo et al., 2016). Schwartz and Herricks (2005), encourage the use of the whole floodplain to perform riverine interventions to effectively promote downstream habitat enhancement for freshwater biota. These may include the re-introduction of side-channels (Hauer et al., 2014; Schwartz and Herricks, 2005) that also assure the lateral connectivity during low flow. Experimental flume-based research has proposed morphological measures to mitigate hydropeaking consequences. For example, lateral refuges (Ribi et al., 2014), substrate heterogeneity (Chun et al., 2011), and alternative cover structures (velocity refuge, visual isolation and overhead cover) (Vehanen et al., 2000) have been studied as refuge alternatives for salmonids during hydropeaking events. With the increasing availability of discharge time-series data and robust hydraulic modelling software, it is also possible to simulate the river hydromorphology and its hydrodynamic conditions (Vanzo et al., 2016), and to create hypothetical scenarios based on fish preferences (Boavida et al., 2017, 2015). By studying these hypothetical scenarios it was possible, for example, to identify beneficial and inadequate morphological measures regarding the geometry of lateral refuges (Almeida et al., 2014) and the form of channel bars on the stranding risk of fish during hydropeaking (Hauer et al., 2014).

Structural measures may include the construction of retention volume structures (e.g. basins or caverns) that temporarily store the discharge peak, attenuating its release in the downstream river system (Bruder et al., 2016; Harby and Noack, 2013). The construction of these structures requires land or river corridor allocations, and demands a thorough evaluation of the economic-ecological benefits. By allowing that a pre-surge state is reached before the maximum discharge is released, these structures assure a gradual adaptation of the downstream biota (Meile et al., 2011; Parasiewicz et al., 1998; Person et al., 2014).

1.4 Physiological and behavioural responses of fish

There is a vast body of research on how fish respond to flow variability (Poff and Zimmerman, 2010). Most of it focuses on ecological responses, such as changes in fish assemblages, which are commonly related with alterations in movement patterns (Murchie et al., 2008). To quantify the effects of simulated hydropeaking in *L. bocagei* and to evaluate the potential of structures as velocity refuges, it was necessary to level down to organism-level responses. The rationale behind this choice was related with the ecological relevance of identifying a mechanistic link between a flow disturbance and the occurrence of a stress response. A stress response is considered an adaptive mechanism that enables fish to cope with any real or perceived stressor with the objective to maintain the homeostatic state (Barton, 2002). In this sense, a stressor can be any external disturbance that threatens the homeostatic state and triggers a complex suite of adaptive responses to regain it, which is the stress response *per se* (Chrousos, 1998; Wendelaar Bonga, 1997) (Figure 1.4).

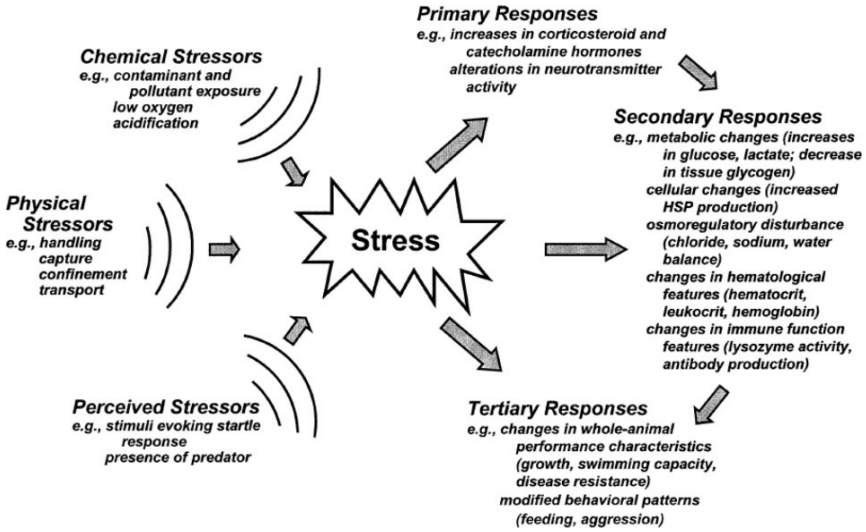

Figure 1.4 Fish responses to stressors. External physical, chemical and other perceived stressors act on the fish organism resulting in physiological changes and other associated effects. These are grouped as primary, secondary and tertiary or whole-animal responses. The primary and secondary responses may directly affect secondary and tertiary responses, respectively (indicated by the responses arrows). Adapted from Barton (2002).

The external stressors act in two successive ways; first, they threaten or disturb the homeostatic state and second, they elicit a coordinated set of physiological and behavioural responses for the organism to cope with that threat (Wendelaar Bonga, 1997). The physiological responses of fish to an environmental stressor are generally grouped in primary, secondary and tertiary responses (Figure 1.4). Primary and secondary responses are pointed out as direct effects, whereas tertiary responses correspond to whole animal performance changes (Barton, 2002) (Figure 1.4). The physiological response to stress involves a coordinated neuroendocrine pathway, initiated with a rapid increase in plasma levels of catecholamines (adrenaline and noradrenalin) (Barton, 2002; Pankhurst, 2011; Wendelaar Bonga, 1997). These short latency responses take within one minute from stimulus perception to response occurrence. This initial response is followed by the activation of the corticosteroid limb of the stress response, via the stimulation of the hypothalamo–pituitary–interrenal (HPI) axis, culminating in the release of corticosteroid hormones (mainly cortisol) to the circulation (Mommsen et al., 1999; Pickering and Pottinger, 1989; Sumpter, 1997; Wendelaar Bonga, 1997). The time-course for the interrenal response to the production of cortisol is variable among species. Scientific evidence demonstrated that the time to detect statistically significant elevations of plasma cortisol in a range of fish species that had credible baseline levels, ranged from 2.5 to 120 minutes (Pankhurst, 2011). The secondary responses to stress can be manifested in: physiological and metabolic adjustments, changes in the cardio-respiratory and in the immune systems, hydro-mineral balance, and cellular responses (Iwama et al., 1998; Mommsen et al., 1999). The rapid elevation of cortisol is accompanied by similarly rapid elevations of other parameters including plasma lactate and glucose (Bracewell et al., 2004). Elevations in plasma glucose are generated initially by catecholamine-mediated glycogenolysis and at later stages, cortisol-mediated gluconeogenesis. Lactate concentrations rise as muscle lactate formed during anaerobiosis is released to the plasma (Begg and Pankhurst, 2004; Mommsen et al., 1999). Tertiary responses, refer to changes in whole-animal individual performance, namely changes in growth, condition, disease resistance, reproduction, survival, and behaviour (Barton, 2002). They may result directly or indirectly from the primary and secondary responses (Figure 1.4).

A behavioural response can be defined as an “individual’s action or response at a given time in a given context” (Mittelbach et al., 2014). Intrinsically associated with a change in the movement behaviour is the mode of aquatic locomotion (Figure 1.5). This represents an adaptive evolutionary trait resultant from natural selection, allowing fish species to adapt to the surrounding habitat and in accordance with specific life-cycle events (Sfakiotakis et al., 1999). The morphological traits intrinsic to each locomotion mode, have enabled fish species to effectively interact with the changes in the flow environment, through locomotion shifts. Those allowed fish to respond to the changing environmental conditions, increasing their success to forage, avoid predators, grow, reproduce, and survive (Kahler et al., 2001; Taylor and Cooke, 2012). For this research, the movement behaviour shifts that could be associated to the simulated hydropeaking conditions were addressed as tertiary or whole-animal responses to flow variability.

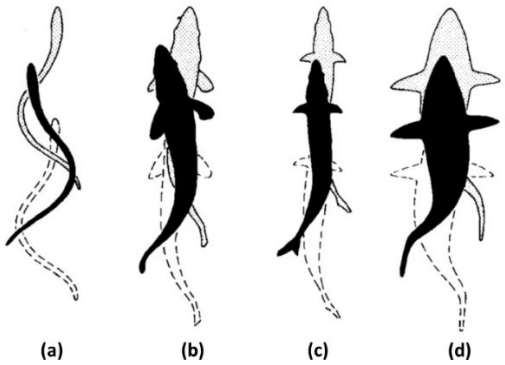

Figure 1.5 Swimming modes and swimming progression illustration associated with body and caudal fin swimming movements from (a) anguilliform, (b) subcarangiform, (c) carangiform and (d) thunniform mode. Adapted from Sfakiotakis et al. (1999).

Group behaviour is particularly relevant while studying fish interactions with changes in the flow environment. In a fish group, the tail beats of the leading fish determine the locomotion of the immediately following fishes (Liao, 2007), and fish in schools save energy irrespective of their spatial position (Marras et al., 2015). Thus, group behaviour may be favourable to save energy (Liao, 2007; Liao et al., 2003; Taguchi and Liao, 2011). Considering that the experimental component of this investigation was conducted in an indoor flume, it was possible to observe both behaviour dynamics, i.e. individual and group movement behaviour. One advantage of conducting behavioural studies under controlled conditions is that it is possible to visualize the behavioural diversity found in nature (Mittelbach et al., 2014). However, it remains challenging to find the ecological consequences of such behaviours (Mittelbach et al., 2014). Understanding this dynamics is essential, as it is decisive for the spatio-temporal distribution of populations, and will likely scale-up to communities and the freshwater ecosystem. For management purposes, understanding smaller-scale movement behaviour provides valuable insight for the concept and design of effective mitigation structures.

Considering an evolutionary perspective, fishes show consistent individual differences in behaviour. However, in an ecological perspective, they also maintain a high degree of behavioural plasticity (Mittelbach et al., 2014). Consistency represents behavioural differences, or traits, that can be maintained within higher levels of ecological organization, because they correspond to adaptive solutions to a stimulus rich environment (e.g. Wolf and McNamara, 2012). Those behavioural and physical adaptations aim at regaining the homeostatic state, improving the chances of the individual to survive (Chrousos, 1998). They represent adaptive responses to challenging events that are not considered stressful *per se* (Chrousos, 1998). The behavioural plasticity is “activated” when fish face unpredictable changes. The extent of the disturbance varies and may result, for example, in fish migrations along the river network to find suitable habitat (Pletterbauer et al., 2014). However, when subjected to severe and prolonged external stressors, the organism may no longer be able to regain the homeostatic state. This results in distress, which corresponds to a chronic response that threatens the overall performance of the organism (Barton and Iwama, 1991; Chrousos, 1998; Wendelaar Bonga,

1997), ultimately extending to traits (fitness, condition, growth) and key life-cycle events (reproduction, survival) (Barton et al., 2002; Pankhurst, 2011).

1.4.1 Fish responses to hydropeaking

Fish responses to hydropeaking range from sub-organismal (e.g. neuroendocrine or metabolic adjustments) (Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012), to changes in life-cycle events (e.g. reproduction, survival, spawning, recruitment) (Burnett et al., 2014; Casas-Mulet et al., 2015b; Kelly et al., 2017; Korman et al., 2011; Puffer et al., 2015). This broad range of responses is mostly related with the spatio-temporal scale of the effects. The study of organism-level responses is to finer spatial scales and short-term effects, whereas the study of populations and communities is to larger spatio-temporal scale effects. Population and community level responses are commonly addressed in riverine conditions. The most conclusive studies usually combine changes in ontogenetic processes with organism-level responses (e.g. Krimmer et al., 2011; Taylor et al., 2012). Reported primary and secondary responses include: elevated corticosteroid levels, metabolic changes in glucose, lactate and oxygen levels, and changes in the muscle activity (Cocherell et al., 2011; Geist et al., 2005; Murchie and Smokorowski, 2004; Taylor et al., 2012). Whole-animal responses associated with hydropeaking include changes in growth, reduced survival rates, or alteration of reproductive success due to obstruction to migration, and alteration in migratory cues (Finch et al., 2015; Fisk et al., 2013; Korman et al., 2011; Korman and Campana, 2009; Vollset et al., 2016; Young et al., 2011). Strictly related with these responses is an associated change in the movement behaviour (Young et al., 2011). This can be manifested in smaller (Krimmer et al., 2011; Scruton et al., 2003; Taylor et al., 2014, 2013; Thompson et al., 2011) to wider spatial-scale movements (Boavida et al., 2017; Burnett et al., 2014; Capra et al., 2017; De Vocht and Baras, 2003; Harvey-Lavoie et al., 2016). However, no changes in the movement behaviour (Jones and Petreman, 2015) or changes that were difficult to interpret (Scruton et al., 2005) have also been reported. This high variability has been mainly attributed to: the presence of velocity refuges, the difficulty to control external variables, and with inter and intra-individual variability (Krimmer et al., 2011; Taylor et al., 2013).

Inter- and intra-individual changes are mostly addressed in experimental flumes (indoor and outdoor). Primary and secondary level responses, such as elevations in corticosteroid levels and changes in glucose have been associated with simulated rapid flow fluctuations in experimental flumes (Flodmark et al., 2002). Because in experimental flumes it is possible to control flow variability, smaller-scale behavioural changes are generally examined. High swimming effort manifested by increased drifting rates (Auer et al., 2017), no changes in social interactions or growth rates associated with down-ramping (Flodmark et al., 2006), and diversified preference patterns towards alternative substrate and refuge configurations under rapid flow fluctuations (Chun et al., 2011; Flodmark et al., 2002; Ribi et al., 2014; Vehanen et al., 2005; Vilizzi and Copp, 2005) have been reported. Although these findings illustrate that

rapid flow fluctuations affect fish performance, some findings were inconsistent and even contradictory. Additionally, it is still challenging to extrapolate a specific change in a flow component (e.g. magnitude or frequency) to population or community-level responses, because these flow changes occur at timescales much shorter than population responses (Taylor et al., 2014).

1.5 Characterization of the hydrodynamic environment

1.5.1 Classical characterization

By characterizing the flow conditions in a river system, it could be possible to identify specific flow features and hydrodynamic stimuli that would trigger different behaviour patterns in fish. However, it is challenging to characterize the hydrodynamic conditions and relate them with specific fish responses at this spatial scale. The existing evidence in such conditions is scarce, but Piper et al. (2015) was able to relate fish movement behaviour responses with hydrodynamic cues associated with the presence of migratory barriers. This constraint could be overcome in experimental flume conditions, where it is possible to control the flow conditions, characterize them in detail and afterwards relate them with fish responses.

Acoustic Doppler velocimetry (ADV) has been an extensively used technology in laboratory conditions to characterize flow fields. It has been broadly used in fishway research (Branco et al., 2013a, 2013b; Silva et al., 2011), or to examine the behaviour of fish under simulated turbulent conditions (Enders et al., 2005, 2003; Goettel et al., 2015). The ADV measures instantaneous velocities for the three orthogonal velocity vectors of the flow (streamwise, *x*; transverse *y*; vertical *z*) at a single-point. These devices are equipped with one transmitter and 2-4 receivers and the sampling volume is located 5 or 10 cm from the tip of the transmitter (Figure 1.6).

The accuracy of the measurements to determine other derived parameters depends on the sampling rate of the equipment and the sampling period (Buffin-Bélanger and Roy, 2005). ADV is easy to use and it accurately measures in 3D over a wide range of velocities (Tuhtan et al., 2017b). In addition, the ADV output can be used to calibrate numerical models, extending their measurements to hypothetical scenarios, and to predict the flow field according to the imposed changes (e.g. Almeida et al., 2017). However, it only performs point measurements up to 2 m depth, and the accuracy of the measurements in the presence of turbulent flows, suspended particles or gas bubbles may be reduced (Chanson et al., 2007; Dombroski and Crimaldi, 2007; Mori et al., 2007; Tuhtan et al., 2017b). The ADV spike noise can be confused with the presence of turbulent components, which may bias the analysis and interpretation of ADV measurements (Tuhtan et al., 2017a). Therefore, it requires data pre-processing to remove spikes (Goring and Nikora, 2002).

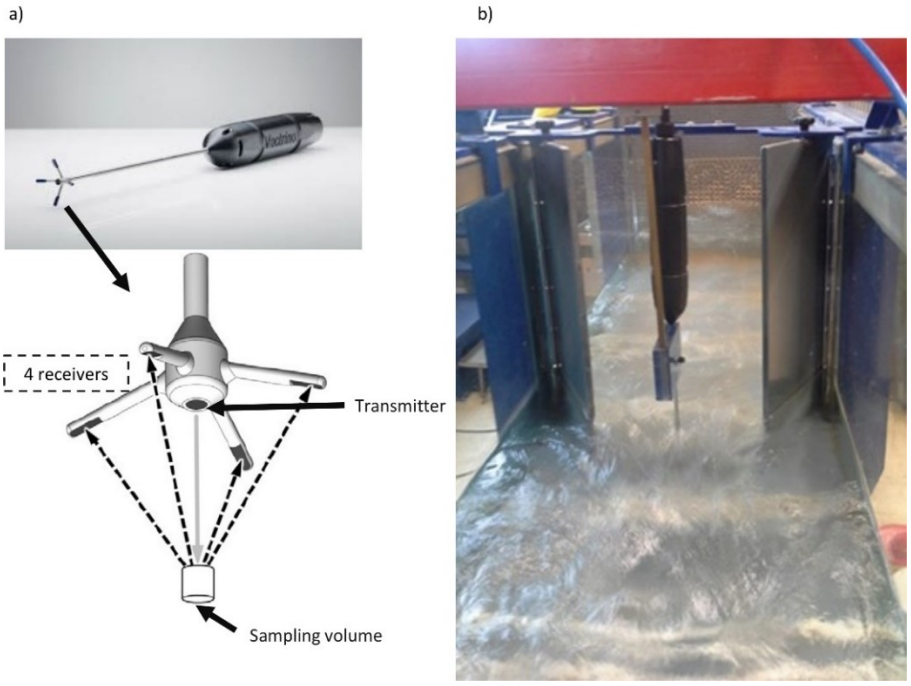

Figure 1.6 ADV equipment used for the experiments in this research. a) down-looking probe and head details [adapted from Nortek (2004) and Sellar et al. (2015)] and ; b) the ADV equipment being used in the experiments during this research.

ADV has been the most extended technology to measure the flow field and to afterwards relate it with fish behaviour (e.g. Fuentes-Pérez et al., 2018; Romão et al., 2017; Silva et al., 2011). For example, the passage success in fishway studies was determined by the combination of velocity and turbulence (turbulent kinetic energy, turbulence intensity, Reynolds shear stress and size of eddies) (Amaral et al., 2016; Fuentes-Pérez et al., 2018; Silva et al., 2015, 2012; Smith et al., 2005), and fish trajectories under turbulence-modified flow fields progressed to consistent turbulence conditions, rather than to regions of lower or higher turbulence (Goettel et al., 2015; Liao, 2007). By combining ADV technology with tools that quantify fish performance (e.g. overall dynamic body acceleration (ODBA), Silva et al., 2015; electromyogram telemetry (EMG), Alexandre et al., 2013) it was possible to relate specific changes in the flow field with fish performance. ADV technology has clearly contributed to understand fish behaviour in different flow conditions. However, it is practically inexistent in hydropeaking studies conducted in experimental flumes (but see Ribi et al., 2014).

1.5.2 Biomimetic characterization

A hydrodynamic stimulus is a combination of water disturbances and pressure fluctuations that are generated by flow, habitat heterogeneity, and the locomotion of aquatic animals (Bleckmann and Zelick, 2009; Kalmijn, 1988). Considering an evolutionary perspective, natural selection has evolved morphological features in fish that are related with the sensorial system. These features are used to detect water movements, pressure fluctuations or both (Bleckmann, 1994), and are highly efficient

considering the fish species life-cycle and habitat (Sfakiotakis et al., 1999). This system, i.e. the lateral line, consists of a mechanosensory system where its smallest functional unit, the neuromasts, are distributed superficially in the fish skin (i.e. superficial neuromast) or in fluid-filled dermal canals (i.e. canal neuromast) (Bleckmann and Zelick, 2009; Dijkgraaf, 1963) (Figure 1.7).

The organization of these sensorial structures is very distinct among species. Superficial neuromasts (SN) can vary from no more than 50 to thousands and occurring in the head trunk and tail fin (Puzdrowski, 1989; Schmitz et al., 2008). Canal neuromasts (CN) can vary in the number, placement and branching pattern of the canals, canal compartmentalization and width, as well as the number, size and placement of canal pores (Coombs and Janssen, 1990; Webb, 1989). The CN layout across diverse species is intriguingly similar and the reason for this similarity is still to be discovered (Ristroph et al., 2015). CN are sensitive to the pressure gradient between neighbouring canal pores. As a consequence, CN respond within their operating range (>0 Hz up to about 150 Hz) approximately in proportion to outside water acceleration (Kalmijn, 1988).

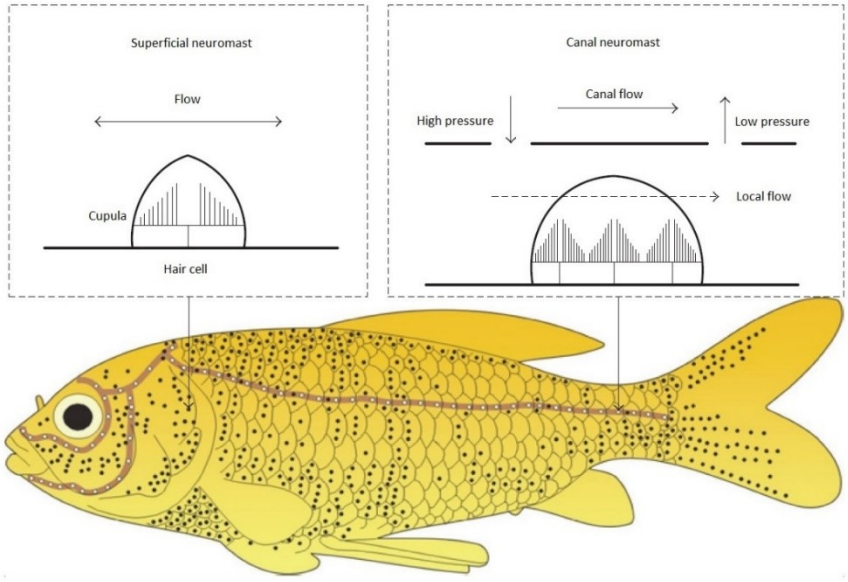

Figure 1.7 Lateral line (light brown line) and neuromasts (black and white dots) of a fish. Black dots and white dots represent approximate distributions of superficial and canal neuromasts respectively. Adapted from Liu et al. (2016).

The processing of external information by the lateral line results in the diversity of behaviour patterns that fish exhibit. The hydrodynamic changes generated by individual fish wakes, provide information about the size, swimming style and swimming speed of the wake generator (Bleckmann and Zelick, 2009; Hanke and Bleckmann, 2004). These self-generated water motions present a useful source of information for interactions between species and individuals of the same species (Bleckmann, 1994, 1986; Montgomery et al., 1997), such as prey detection or predator avoidance (Bleckmann et al., 1989; Coomb et al., 2012; Coombs et al., 2001; Kanter, 2003). In addition, using the lateral line fish are able to

detect the vortices generated by instream structures. The vortices can be used to reduce their swimming costs (Liao, 2006; Liao et al., 2003), optimize rheotactic behaviour (Montgomery et al., 1997), or detect and determine the direction of moving objects (Vogel and Bleckmann, 1997).

Thus, the morphological characteristics of this system ensure the distributed sensing capacity of fish, playing a determinant role in fish behaviour. It could be possible to determine how the flow field created by flow fluctuations and the presence of structures would affect their swimming behaviour if we could understand how local hydrodynamic changes are interpreted by this system. Researchers have tried to understand this sensory system by developing artificial analogues: the artificial lateral lines. Liu et al. (2016) reviewed the use of artificial lateral lines, their design and fabrication principles, applicability, and hydrodynamic detection ability. For example, cylinder-shaped tubes with distributed artificial neuromasts have accurately detected hydrodynamic stimulus, and the presence of organisms in motion (Yang et al., 2010). The algorithm developed by these authors has been successfully applied in other artificial line systems (Liu et al., 2009). Based on this evidence, artificial lateral lines seem to be able to detect hydrodynamic changes. However, the causality between small spatial-scale movement behaviour shifts and local hydrodynamic changes using artificial lateral lines is just emerging (Fuentes-Pérez et al., 2018; Tuhtan et al., 2017b). In contrast to conventional flow measuring technologies that only perform point velocity measurements (e.g. flow meters, ADV, propellers), artificial lateral lines measure pressure arrays around the body of the probe. Additionally, this technology is able to accurately measure in highly turbulent and complex flows, where ADV measurements can be difficult (Shizhe, 2014).

For this research a fish-shaped artificial lateral line probe was used (described in 4.1.3). This probe consists of a streamlined fish-shaped body with six differential pressure sensors and is able to measure the spatiotemporal interactions of the flow with the body in terms of pressure (hydrostatic and dynamic pressures). Thus, it simulates the distributed sensing capacity of the canal neuromasts (Tuhtan et al., 2017b). This probe is able to measure at the same rates as the natural sensing frequencies of fish (10 to 100 Hz) (Fuentes-Pérez et al., 2015), mimicking, at some extent, their sensing capacity.

1.6 The Iberian barbel

Although cyprinids are rarely considered in hydropeaking studies, Cyprinidae is the most representative fish family in freshwater ecosystems (Nelson et al., 2016). The intrinsic biogeography of the Iberian Peninsula and the simultaneous high seasonality of its river networks, particularly evident in rivers affected by Mediterranean climate, have resulted in a high level of endemism, with highly restricted species distribution patterns and highly vulnerable to habitat disturbances (Clavero et al., 2004; Collares-Pereira et al., 2000; Santos et al., 2017). For this research, the selected cyprinid species was the Iberian barbel, *Luciobarbus bocagei* Steindachner, 1864 (*Figure 1.8*) (hereafter, *L. bocagei*). This

is a potamodromous fish, endemic to the Iberian Peninsula, and widely distributed in the northern and central river catchments of Portugal (Lobón-Cerviá and Fernandez-Delgado, 1984; Oliveira et al., 2002).

Figure 1.8 Iberian barbel, *Luciobarbus bocagei* Steindachner, 1864. Photograph taken during the experiments conducted at the Laboratory of Hydraulics, IST.

This species has a wide spectrum of velocity preferences that change during its ontogeny (Kottelat and Freyhof, 2007). Although primarily considered a limnophilic species, preferring mostly slower water velocity habitats (Oliveira et al., 2007, 2002) it has also been described as rheophilic (Ferreira et al., 2007a) and eurytopic (Lobón-Cerviá and Fernandez-Delgado, 1984). Fingerlings and juveniles are predominantly rheophilic (Martínez-Capel and García de Jalón, 1999) and in the reproductive season, the adults prefer faster water currents to perform upstream migrations to spawn in sandy and gravel bed substrates (Rodriguez-Ruiz et al., 1992). Outside these life-cycle events, *L. bocagei* tends to be limnophilic.

Morphologically, *L. bocagei* has a fusiform and laterally compressed body, with lower thick lips with two pairs of barbels, and a bony last ray of the dorsal fin (Kottelat and Freyhof, 2007). As for its swimming mode, it is characterized by an undulatory subcarangiform swimming mode where the amplitude of the undulations is limited anteriorly, and increases only in the posterior half of the body (Sfakiotakis et al., 1999) (Figure 1.5). This locomotion mode ensures that this species is slower than, for example, carangiform swimmers. However, by using the posterior half of the body for propulsion, it enables them to be more efficient in the turning and accelerating abilities (Sfakiotakis et al., 1999).

The inherent ecological value of this species is undeniable, given its specific habitat requirements during ontogeny, the diversity of velocity preferences, and its flexible swimming mode.

1.7 Thesis motivation

It is generally recognized that the flow regime is determinant for the ecological integrity of river ecosystems (Lytle and Poff, 2004; Poff et al., 1997). Hydropower plants and their operation schemes

produce drastic alterations in the downstream flow regime, with extensive impacts on downstream fish communities. Those impacts are manifested by abundance shifts which result from adaptations, migrations or local extinctions (Comte et al., 2013; Comte and Grenouillet, 2013). As the number of hydropower plants that are planned or under construction (Zarfl et al., 2015) and the global hydroelectricity production (IHA, 2018) are expanding, it is likely that the structure and function of these vulnerable systems will further deteriorate (Hoekstra and Chapagain, 2007; Poff and Zimmerman, 2010).

Given this escalating trend for peak hydroelectricity it is necessary to effectively identify its challenges, to understand its impacts, and to propose guidelines to mitigate its consequences. As it is impractical to propose operational measures that are cost-effective to hydropower plant managers, or to suggest the removal of these infrastructures, these solutions are rarely selected. Thus, the main challenges for researchers are to identify the flow changes that result in quantifiable responses and, together with hydropower plant managers, to propose morphological mitigation solutions or scientifically grounded flow standards.

The relevance of this thesis is grounded in the alarming aspect that rivers will increasingly continue to be affected by hydropeaking. Thus, it is necessary to scrutinize the effects of downstream flow regime alterations due to hydropower production to fluvial fish, and to propose alternative mitigation guidelines that are favourable to hydropower plant managers, while protecting the downstream fish communities. In this research the effects of rapid flow fluctuations associated with hydropeaking were investigated for *L. bocagei* in an indoor flume, and the potential of artificial structures as a morphological mitigation measure was evaluated. Taking advantage of the benefits of experimental flumes, it was possible to examine smaller-scale behaviour patterns of fish in relation to flow fluctuations. With this analysis it was expected to get a whole representation of the fluid-structure-fish system. This inter- and intra-individual approach is grounded in the rationale that any disturbance affecting the individual has the potential to be magnified to populations, communities and ultimately the river ecosystem (Amiard-Triquet, 2009). With the adopted methodological approach it was expected to: (1) attribute a fish response to a specific flow change, (2) determine whether the severe flow changes result in behavioural adjustments or present a real threat to fish, and (3) propose guidelines for the concept and design of mitigation measures.

1.8 Main objectives

The scientific evidence for a cause-effect relationship between flow variability and a specific fish response was scarce before the materialization of this research. Most studies addressed changes in fish assemblages in their natural habitat. Hence, it was challenging to find such causality. To identify that mechanistic link it is necessary to level down to lower ecological units, specifically to the organism-level. The first general objective of this research, corresponding to the first part of the thesis, was to provide

the scientific community a comprehensive overview of the existing evidence for organism-level responses of fluvial fish to flow variability. The gathered insight provided knowledge to select potential fish responses as biomarkers for flow variability for future research in both natural and modified flow conditions. For the purpose of this thesis, this review was the starting point for the experimental research to be conducted, particularly regarding the choice of physiological responses to rapid flow fluctuations.

To date, the conceptualization of habitat mitigation measures to hydropeaking consequences for cyprinids and their implementation in natural conditions is practically inexistent. To address the causality between flow variability and a specific fish response, and to minimize the effects of confounding variables that naturally occur in the river ecosystem, the rapid flow fluctuations associated to hydropeaking were simulated in an indoor flume located at the Laboratory of Hydraulics at IST, University of Lisbon. In this sense, the general objective of the second part of this thesis was to quantify the behavioural responses of *L. bocagei*, to simulated hydropeaking, and to conceptualize refuges as a morphological mitigation measure. A multidisciplinary approach was adopted, which included the quantification of physiological responses and movement behaviour frequency, hydraulic modelling, and fluid-body interactions. The specific objectives of this thesis (first and second part) were to:

- Review the existence and utility of organism-level responses that may be used as biomarkers to assess the effects of flow variability in fluvial fish;
- Examine the effects of simulated base-flow and hydropeaking conditions on the physiology and movement behaviour of *L. bocagei* at an indoor flume equipped with artificial structures as potential velocity refuges;
- Conceptualize alternative structures and spatial arrangements as mitigation measures to hydropeaking consequences for *L. bocagei*;
- Analyse whether *L. bocagei* use the available structures equally under simulated base-flow and hydropeaking conditions;
- Analyse the swimming behaviour of *L. bocagei* under simulated hydropeaking conditions in the presence or absence of structures;
- Identify the hydropeaking conditions that have the lowest and the strongest effects in *L. bocagei*, based on the physiological and behavioural responses together with the characterization of the hydrodynamic conditions;
- Find critical thresholds of local hydrodynamic variables for *L. bocagei*, according to the physiological and behavioural responses;
- Propose alternative structures as velocity refuges, considering the final insights from this research.

1.9 Methodological approach

For the first part of this research a systematic bibliographic review was conducted. A search string, consisting of relevant keywords in the topic of flow variability as an organism-level stressor for fluvial fish, was inserted in the Thomson and Reuters *Web of Science* database. To identify such relationship, a comprehensive analysis of the literature was conducted. The literature that was analysed included natural and anthropogenic flow variability, and *in situ* and laboratory studies. The organism-level responses considered for the analyses were the primary, secondary and tertiary-level responses.

For the second part of this research, the classical scientific method that characterizes natural sciences was used. Starting with the hydropeaking problematic and following with the formulation of the hypotheses, the research consisted of systematic observations, measurements, formulation of new hypotheses and new measurements, thus depicting a full experimental structure.

The experiments were conducted at the indoor flume which intended to mimic a river reach. Under these conditions, it was possible to control the flow changes, and to isolate the effect of potential confounding variables. The indoor flume has a false bottom where it was possible to install different structures that were conceptualized as potential refuges. The effects of hydropeaking were firstly studied in the presence of lateral deflectors, which are broadly used in restoration actions (Pretty et al., 2003) (see 3.1.3 and 3.2.4). As new questions emerged, instream structures were conceptualized in the last experiment (see 4.1.3). A diverse set of flow events was tested, where magnitude, peak duration, peak frequency and event duration changed. For all experiments, the base-flow conditions consisted of a continuous 7 $l.s^{-1}$ flow event. The peak discharges tested were 20, 40 and 60 $l.s^{-1}$. The choice of these discharges changed in the progress of the experiments.

To examine the effects of hydropeaking and the presence or absence of structures for *L. bocagei*, a novel and multidisciplinary methodological approach was used. It combined fish responses at the organism-level and whole-animal performance, with a detailed characterization of the hydraulic and hydrodynamic conditions created in the flume. To find out whether the rapid flow changes presented a stressor for *L. bocagei*, blood glucose and lactate were selected as secondary level responses of the HPI axis. Whole animal performance was analysed by quantifying specific movement behaviour metrics related with the flow events and the refuges tested. In this sense, refuge use and swimming activity metrics likely to be visible under hydropeaking conditions were quantified. To characterize the hydraulic conditions and the hydrodynamic environment two technological approaches were used: (i) point velocity measurements using ADV, followed by a calibration to a theoretical 3D hydrodynamic model (see 3.1.3 and 3.2.4), and (ii) a novel technology, the LLP, which measures fluid-body interactions (see 4.1.3).

With the findings from this multidisciplinary approach it was possible to propose operational alternatives and morphological measures to mitigate the effects of hydropeaking for *L. bocagei*. Hopefully these may be the basis for future research and followed by hydropower plant managers.

1.10 Thesis structure

This thesis is organized in 5 chapters. Chapter 1 is the "Introduction" to the thesis. Within this chapter, the classical frameworks and concepts that were the grounds for the following chapters are introduced. In addition, the necessary background that resulted in new hypotheses testing and the objectives that were proposed are exposed. The reader will be able to understand essential concepts related with flow variability, its role for freshwater fish, and the consequences of its natural and anthropogenic fluctuations. Afterwards, the motivation behind this research is revealed. The problematic to be addressed and its relevance for the scientific community and freshwater managers are exposed. The general and specific objectives are enumerated, as well as the methodological approach that was used to test the hypotheses formulated. Finally, the thesis structure is presented.

Chapter 2, "Finding cause-effect relationships between flow variability and fish responses" corresponds to the first part of the thesis. The general objectives of this study were to provide a comprehensive overview of flow variability as an organism-level stressor for fluvial fish, by finding a mechanistic link between flow variability and a quantifiable fish response based on previous scientific evidence. This chapter had two essential roles: (i) to present experimental approaches and potential biomarkers that demonstrated to be valid to address the effects of natural or anthropogenic flow variability to fluvial fish, (ii) to select the physiological responses of *L. bocagei* to be quantified in the second part of this research. This chapter comprises a self-contained review article.

Chapters 3 and 4 correspond to the second and experimental part of this thesis. Chapter 3*, "*Responses of Iberian barbel to simulated hydropeaking: the role of lateral refuges" demonstrates the effects of simulated hydropeaking conditions on *L. bocagei*. Additionally, it presents the role of deflectors, with distinct configurations (i.e. meandered – see 3.1.3, and one-sided - see 3.2.4), as potential mitigation solutions to hydropeaking consequences for *L. bocagei*. To achieve the objectives proposed for this chapter *L. bocagei* responses were quantified, and the hydraulic conditions created by the flow event and the deflector configuration were characterized using ADV. The purpose of this chapter was to present the effects of hydropeaking for *L. bocagei* in the presence of refuges, to investigate the differences in *L. bocagei* responses according to the presence and absence of those refuges, and to identify the rapid flow changes that produced the most and the least visible responses. The deflectors and their spatial arrangement were proposed as a mitigation measure to the consequences of hydropeaking. The use of the multidisciplinary approach aimed at providing a more robust interpretation of the results to efficiently define whether the structures might represent effective

mitigation measures in rivers affected by hydropeaking, and to propose hydropeaking conditions that least affected *L. bocagei*. This chapter corresponds to two distinct experimental campaigns and comprises two self-contained research articles (see 3.1 and 3.2).

Chapter 4, "Defining mitigation measures to hydropeaking supported by fluid-body interactions", resulted from the formulation of new hypotheses based on the results from the previous chapter. In particular, to evaluate the effects of the hydropeaking event that produced the most evident fish responses, corresponding to the single-step peak event where the highest discharge was tested. In addition, new instream structures to serve as velocity refuges were conceptualized. The changes in the local flow field created by the flow event and the instream structures were characterized using the LLP. This was the first study that used the LLP to relate movement behaviour patterns of fish with local hydrodynamic changes. In this sense, there are no studies to make comparisons. This chapter corresponds to the last experimental campaign and comprises one self-contained research article (see 4.1).

Although chapters 2, 3, and 4 are identical to the published or soon-to-be published versions, substantial editing differences exist. To make the exposition of the work more fluid, the locations and size of figures and tables have been changed.

Chapter 5 summarizes the major findings of this investigation, the challenges and limitations that were found during the experimental work, its practical applicability, and discusses future research considering the hydropeaking problematic.

2

Finding cause-effect relationships between flow variability and fish responses

"An individual human existence should be like a river – small at first, narrowly contained within its banks, and rushing passionately past rocks and over waterfalls. Gradually the river grows wider, the banks recede, the waters flow more quietly, and in the end, without any visible break, they become merged with the sea, and painlessly lose their individual being".

Bertrand Russel

2.1 Is there evidence for flow variability as an organism-level stressor in fluvial fish?

Review article published in the *Journal of Ecohydraulics*; Received: 29 July 2016 / Accepted: 30 Dec 2016

Costa, M. J., Lennox, R.J., Katopodis, C. and Cooke, S.J. (2016). Is there evidence for flow variability as an organism-level stressor in fluvial fish? *Journal of Ecohydraulics*: 2 (1), 68-83.
DOI: 10.1080/24705357.2017.1287531

2.1.1 Abstract

Flow regime is a fundamental driver in fluvial ecosystems, shaping habitat structure and biodiversity, and sustaining ecological integrity. Fish respond to flow fluctuations but whether aspects of flow variability represent organism-level stressors is poorly understood. To find existing evidence of fluvial fish organism-level responses to flow variability (natural or anthropogenic), and whether it resulted in quantifiable stress (i.e., departure from homeostasis), we reviewed literature on the physiological responses of fish exposed to flow. Among 58 articles that we identified to be relevant to the research question, 40 reported whole-animal responses (tertiary responses to stress), 37 reported blood and tissue level changes (secondary responses to stress) and 18 reported neuroendocrine changes (primary responses to stress), exclusively or combined. Whole-animal responses (e.g. growth or disease resistance) were more commonly assessed due to their broader use in population and community dynamics studies. Due to their long-term character it was difficult to isolate flow variability as the only stressor and to understand the underlying mechanisms that culminated in a stress response. Our review indicates that flow variability can be a stressor for fish but it remains unclear if events such as floods or hydropeaking are inherently stressful per se. More experimentation is needed to find out if flow variability presents a stressor to fluvial fish, what thresholds trigger a stress response and to better understand the relative role of the different flow components. This knowledge can be used to define robust stress biomarkers (particularly for field studies) and propose adequate flow thresholds.

Keywords

Flow variability; physiological responses; natural flow regime; flow; stress; fluvial fish

2.1.2 Introduction

In an ecological context, flow regime is a key driver of fluvial ecosystems shaping and controlling habitat structure, biodiversity composition and inherent ecological processes (Allan and Castillo, 2007; Katopodis, 2005; Naiman et al., 2008; Poff et al., 1997). The temporal and spatial character of flow components, including magnitude, frequency, duration, timing, and rate of change (Olden and Poff, 2003; Poff et al., 1997) affect population and community dynamics, biotic interactions and short and long-term biological processes (Freeman et al., 2001; Gasith and Resh, 1999; Humphries et al., 1999; Krimmer et al., 2011; Lytle and Poff, 2004; Ugedal et al., 2008; Young et al., 2011). Flow is inherently variable, driven by geomorphology, landscape features (biotic and abiotic) and atmospheric processes (Black, 1996; Brown et al., 2011b; Gordon et al., 2004; Katopodis and Aadland, 2006). Increasingly, anthropogenic pressures are contributing to changes in natural hydrological regimes (Vörösmarty et al., 2010) and are even aggravated in modified riverine systems. Examples of changes in the natural hydrograph include the release of artificial discharges from hydropower dams for electricity production (e.g. hydropeaking, Poff et al., 2003; Young et al., 2011), the extent and constant alteration of impervious surfaces in urban centres (Walsh et al., 2005), water abstraction for irrigation (Haddeland et al., 2006), and the installation of stormwater management facilities that rapidly move water off the landscape (Wanielista and Yousef, 1993). Being constantly subjected to human alterations, pristine fluvial ecosystems are now rare (Allan and Castillo, 2007). With the increasing demand for more efficient energy, food and clean drinking water, the consequent landscape alterations and the influence of climate change it is anticipated that river flows could be even more variable in the future (Arnell, 1996; Hoekstra and Chapagain, 2007; Rijsberman, 2006). As a consequence, the continuous alteration of flow regime worsens the threats to biodiversity of rivers at a global scale (Dudgeon et al., 2006; Nilsson et al., 2005). Not surprisingly, freshwater fish are among the most threatened taxa on the planet (Bruton, 1995).

Given the global interest in river restoration (e.g. Katopodis and Aadland, 2006) and the ever increasing identification of optimal flow regimes in regulated rivers (e.g. Katopodis, 2005), it is not surprising that there is a vast body of research on how a variety of organisms, but especially fish, respond to flow dynamics (Poff and Zimmerman, 2010). However, most of the research to date has focused on responses of fish populations and changes in assemblages (Murchie et al., 2008) rather than adopting a more mechanistic approach to understand if and how flow may result in organism level stress. There already exists a rich history of research on how fish respond to different natural and anthropogenic stressors, such as temperature, salinity, hypoxia and biotic and social interactions (e.g. predation, aggressive behaviour, reviewed Barton, 2002; Fry, 1971; Wendelaar Bonga, 1997). Any real or perceived variation in these factors threatens the individual homeostasis and results in a stress response that acts as an adaptive mechanism to restore it (Barton, 2002). Increasing the intensity of the stressor may

compromise the organism capacity to respond with an adaptive mechanism, having consequent deleterious effects on the fish well-being (Barton, 2002). The stress response begins by the recognition of a real or perceived threat by the central nervous system. Afterwards, a cascading set of endocrine responses involving chromaffin tissue and the hypothalamic-pituitary-interrenal axis is triggered, resulting in the physiological responses to stress (Barton, 2002). These have been broadly grouped in primary and secondary, the first being associated to an initial neuroendocrine response, including changes in circulating stress hormone concentrations (adrenaline, noradrenaline and cortisol; Mazeaud et al., 1977), and the second to physiological adjustments in metabolism, including changes in energetic metabolism, hydro-mineral balance, and cardiovascular, respiratory and immune function (Barton, 2002). Finally, tertiary responses include changes in whole-animal performance characteristics, like growth, reproduction, disease resistance and behaviour that may result directly or indirectly from primary and secondary responses (Barton, 2002). These are generally indicative of long-term (chronic) stressors in opposition to the more immediate primary and secondary responses.

Flow variability, here referred to the natural or anthropogenic changes in flow that have the potential to produce organism level changes, is rarely considered or even mentioned in the context of environmental or ecological physiology. However, with the increasing alteration of flow regimes (Nilsson et al., 2005), it is necessary to understand the effects of these changes in organism level mechanisms to identify the extent to which these mechanisms are affected by flow and to effectively predict responses to flow variability (Nislow et al., 2004). It is essential to understand the complexity of the adaptive mechanisms to flow variability, because presumably the patterns in population level processes are driven by individual level differences in condition, health, energy levels, and physiological status (Calow, 1989; Calow and Forbes, 1998; Maltby, 1999; Pankhurst, 2011; Young et al., 2011) The kinetics of cortisol is an example of a physiological mechanism that is broadly used to study the degree of stress experienced by fish (Barton and Iwama, 1991; Wendelaar Bonga, 1997). With proper experimental protocols it is possible to quantify the circulating levels of cortisol, either they are resting levels (Gamperl et al., 1994) or after being affected by an external stimulus (e.g. swimming exercise, Zelnik and Goldspink, 1981). Although we acknowledge the ecological effects of flow variability on fish in the river ecosystem (Poff and Zimmerman, 2010), explaining if and how the severity of natural or modified flow conditions presents a challenge to fish is poorly understood. There are examples of experimental protocols linking swimming activity with fish stress physiology (e.g. Young and Cech, 1993; Young and Cech Jr, 1994a, 1994b; Zelnik and Goldspink, 1981); however they do not establish a relationship with the flow conditions experienced in the natural environment. Understanding the complexity of the adaptive mechanisms to flow variability would provide improved guidance for establishing biologically appropriate environmental flow solutions, whether using physiomimetic (i.e. nature-mimicking) approaches or habitat modelling (Katopodis, 2005; Katopodis and Aadland, 2006). To that end, this paper provides an overview of the evidence for organism level responses of fluvial fish to flow fluctuations (natural or modified) and whether or not they produce a physiological response that can be

measured and scaled to populations, communities and the ecosystem. The fluvial ecosystem will be the focus of this review because its ecological integrity depends on the dynamic equilibrium of its dimensions (longitudinal, lateral, vertical and temporal) and any disturbance, either anthropogenic or not, has the potential to disrupt the river continuum and to affect all ecological units, from organism to ecosystem (Poff et al., 1997; Vannote et al., 1980). Any study that has focused on fish species that spend part of their life-cycle in freshwater (both anadromous and catadromous species) was considered.

2.1.3 Research approach

To find relevant literature on the physiological responses of fluvial fish to flow variability, we selected the electronic database Thomson Reuters Web of Science (https://apps.webofknowledge.com) and searched for articles published until the end of 2015. We first listed a set of possible keywords that in a second phase were combined and used as search strings in Web of Science. In the end we used the following search strings: (1) (flow OR flow NEAR/5 regime) + (fish*) + (stress* OR physiol*) NOT (marine OR estuar * OR aquaculture); (2) (flow* OR discharge OR 'water level') + ('stress* response*' OR physiol*) + (stream* OR river*) NOT (aquaculture OR marine OR estuar*) + (change* OR fluctuat* OR modif* OR reduct* OR regime* OR pulsed OR regulat*); (3) (flow* OR discharge OR 'water level') + ('stress* response*' OR physiol* OR stress*) + (stream* OR river*) NOT (aquaculture OR marine OR estuar*) + (change* OR fluctuat* OR modif* OR reduct* OR regime* OR pulsed OR regulat*); refined by (fish*); (4) = to (3) and refined by: (fish* OR cyprini* OR salmonid*). For each search string, we screened the relevant titles and abstracts and for each article we screened the first 50 titles and abstracts of related articles. We only considered peer-reviewed documents. Additionally, we only selected English written documents. We have included relevant studies that considered the effects of flow variability on both growth and condition of fish, even if not indicated at any point of the study as a stressor. The Web of Science search results were quantitatively analysed to identify temporal and geographical trends, quantify the most used flow variability-organism level responses, and determine the most common focal fish taxa to understand the adaptive mechanisms associated with the flow change. Flow-stress responses were classified in Primary (I), Secondary (II) and Tertiary (III), based in Barton (2002) terminology (Table 2.1). The qualitative analysis of how fluvial fish responded to flow variability at an organism level, focused in natural and modified flow regimes, considering the role of flow regime as a driver of fluvial ecological integrity and the increasing severity of flow regulation.

Table 2.1 Fish responses to stress analysed in this review (adapted from Barton 2002), designated sub-categories and examples of studied responses found in the collected literature.

TYPE OF RESPONSES TO STRESS Sub-category	Examples of possible organism level responses (changes)
PRIMARY RESPONSE (I)	
Corticosteroids	Blood cortisol
Catecholamine	Epinephrine, Norepinephrine
SECONDARY RESPONSE (II)	
Metabolic changes	Blood glucose; blood lactate; tissue glycogen; lipid metabolism; plasma pH
Cardio-respiratory system	Oxygen metabolism
Immune System	Hematocrit; antibody production
Ion-osmoregulation	Chloride; osmolality; potassium; sodium
Muscle activity	Physiological telemetry (e.g. electromyogram activity)
TERTIARY RESPONSE (III)	
Fitness	Lipid content
Reproductive success	
Growth	Otolith striping; body condition
Disease resistance	
Swimming behaviour	Aggression; foraging; activity
Egg and larvae survival	

2.1.4 Findings in flow variability-stress responses

Temporal and geographical trends according to flow regime studied

The use of physiological responses as fish performance indicators became more relevant since the emergence of the natural flow regime paradigm and the recognition of its role as a key driver of the river ecosystem (Poff et al. 1997; Figure 2.1).

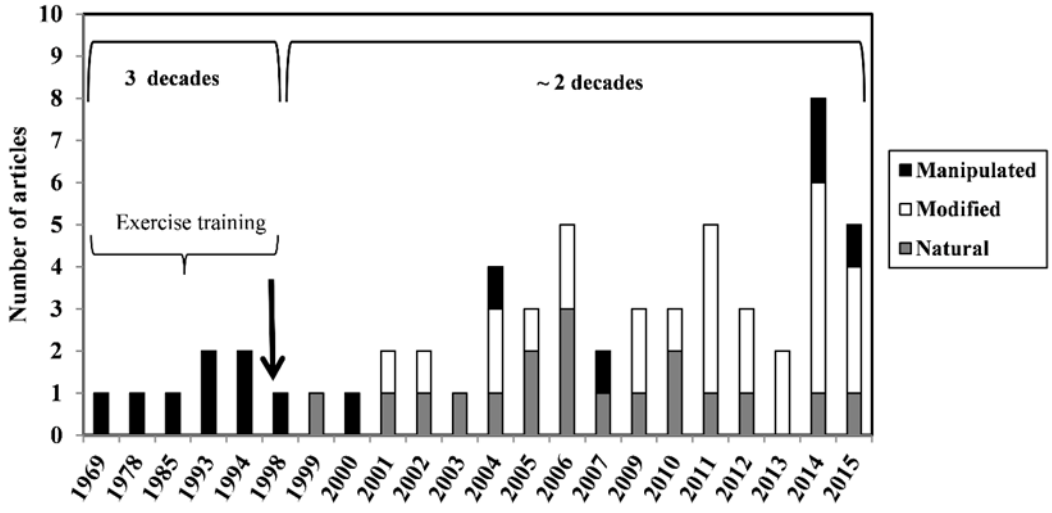

Figure 2.1 Temporal trends according to types of flow regime investigated. Manipulated – the research was conducted in laboratory conditions where flow was manipulated either in tanks, flumes or respirometers; Modified – the research was conducted in natural or controlled conditions that simulated altered flow regimes; Natural – the research was conducted in the natural environment under natural flow regime or in controlled conditions simulating the natural environment. The arrow indicates the year were "natural flow regime paradigm" was first described by Poff et al. 1997.

This novel approach emerged given not only the importance of flow regime as a driver of the river ecosystem but also given the increasing extent of river regulation. This focus on the effects of flow dynamics at the individual level could be attributed to the need in understanding individual level mechanisms to flow changes and how they would affect higher ecological units (Bunn and Arthington, 2002; Poff and Zimmerman, 2010).

We identified 58 relevant articles (Supplement 1) reporting whole- and sub-organismal responses according to flow variability features. The first studies reporting organism level effects of flow variability were conducted in laboratory conditions where flow was manipulated to perform exercise training. This research approach aimed at optimizing fitness of hatchery-reared fish for conservation purposes, rather than attributing to flow variability a stressor effect (Burrows, 1964; Farlinger and Beamish, 1978; Woodward and Smith, 1985; Young and Cech, 1993; Young and Cech Jr, 1994a, 1994b, 1993), Figure 2.1). This line of investigation was prominent for three decades (Figure 2.1). Since then, fish fitness optimization by exercise training has been reviewed (Davison, 1997, 1989) and its efficiency has been subsequently questioned (Brown et al., 2011a). From the amassed literature, it was evident that most of the research focusing on organism level responses occurred in the USA (28 %) and Canada (27%), followed by Norway (14%), UK (9%) and China (9%). These results are not surprising because China ranks first among global hydropower producing countries, but lacks English written articles, USA and Canada rank third and fourth place, respectively, and Norway ranks seventh (first in Europe) (IHA, 2015). Notably, 31% of the USA-based studies focused on exercise training and took place before the natural flow regime paradigm was described.

Exercise training studies and respirometry studies revealed that fish velocity preferences were a determining factor influencing whole- and sub-organismal responses. Exercised (trained) salmonids showed accelerated maturation timing compared to fish in slack water (Patterson et al., 2004), and poecilids improved their sustained swimming performance (Sinclair et al., 2014). At a sub-organismal level, salmonids trained at low velocities after a bout of exhaustive exercise, experienced lower levels of plasma cortisol levels than fish recovering in still water (Milligan et al., 2000). However, centrarchids training at low-velocity after exhaustive exercise exhibited signs of physiological disturbance, absent in still water (Suski et al., 2007). Respirometry studies provided valuable insight on how different flow conditions associated with other environmental factors (e.g. predation, restricted ration, pollution) resulted in intensified competition with trade-offs between growth, feeding and swimming activity (Gregory and Wood, 1998), exerted selective pressure on swimming performance, morphology and plasticity (Fu et al., 2015, 2014, 2013), and increased tissue DNA damage due to exhaustive exercise (Aniagu et al., 2006).

Trends in organism level responses investigated

From the 58 collected articles, 19 quantified a stress response directly linked with a flow feature in study (Figure 2.2). Conversely, in 39 of the collected articles there was not an evident cause-effect relationship between flow variability (natural or modified) and an actual measurable stress response. While focusing on unknown aspects of ecologically important species for conservation purposes, or on the metabolic costs associated with swimming activity, these studies provided fundamental knowledge about fish physiology by using novel approaches (e.g. exercise training, Burrows, 1964; Young and Cech, 1993; Young and Cech Jr, 1994a) and tools (e.g. respirometry, Gregory and Wood, 1998). Thus, among the 31 articles that quantified a primary and/or secondary response (Figure 2.2, white bars), 12 aimed at understanding fish physiological performance under manipulated flow conditions that were not necessarily related with a particular hydrological regime.

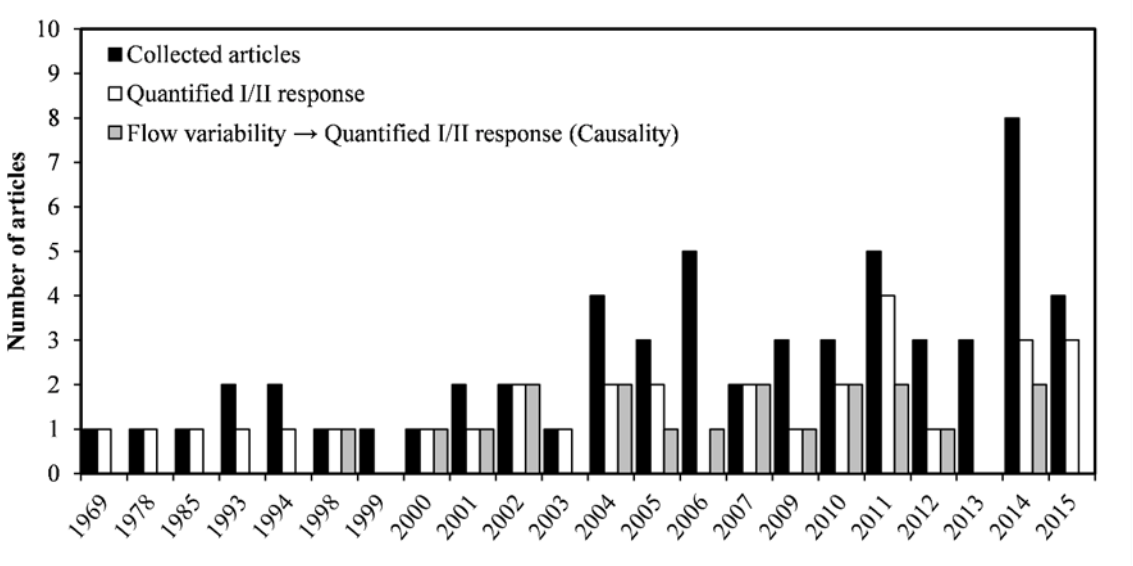

Figure 2.2 Temporal trend according to fish sub-organismal primary (I) and/or secondary (II) responses. From the 58 collected articles (black bars), 31 quantified a sub-organismal response, primary (I) and/or secondary (II), related to a flow feature variability (white bars) and of those, 19 found causality between flow variability (natural or modified) and a sub-organismal response (grey bars).

From the articles with no evident link between a flow variability feature and a stress response, 20 addressed exclusively tertiary responses (

Figure 2.3). This is probably rationalized by 1) the difficulty in establishing causality between flow disturbance and a sub-organismal response in long-term studies, 2) the difficulty in measuring them in the natural environment and 3) the transient nature of primary and secondary responses. Whole animal responses, such as growth and survival, are more commonly used to monitor fish population dynamics and community structure and to assess the ecological status of the fluvial ecosystem. In opposition to

primary and secondary responses, sampling protocols are not indicated to affect whole animal responses and are easy to obtain.

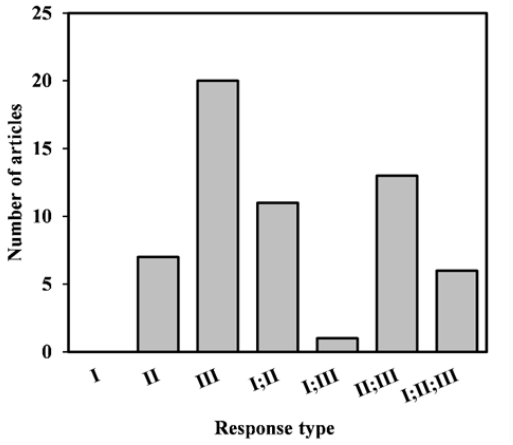

Figure 2.3 Number of assembled articles according to studied response type. (I) Primary response to stress; (II) Secondary response to stress; (III) Tertiary response to stress.

However, due to other confounding environmental factors, such as water temperature or water dissolved oxygen, and to biological factors, such as inter-specific interactions (e.g. predation, Sloman et al., 2002, 2001), attributing causality to flow variability could result in biased assumptions. Thus, to analyse short-term flow disturbances and to directly establish a link between an organism response and a flow feature, primary and/or secondary stress responses were preferred, being analysed in 38 articles. This is justified by the celerity of getting measurable responses, allowing causality with the flow disturbance of interest. Notably, most of these studies focused on the ecologically and economically important salmonid family, with rainbow trout (*Oncorhynchus mykiss*) (7 of 58), brown trout (*Salmo trutta*) (7 of 58) and Atlantic salmon (*Salmo salar*) (7 of 58) as the most studied species. In addition to this, a significant amount of research has already been directed to identify flow requirements for specific life-cycle stages in salmonids (Signore et al., 2016). These fundamental studies can be used as references for future studies focusing on the potential stressful effects of flow variability in salmonids inhabiting highly modified fluvial ecosystems.

2.1.5 Responses in natural flow conditions

The variability inherent to natural flow regime was investigated in 18 of the 58 collected articles with droughts (Balcombe et al., 2012; Harvey et al., 2006; Maceda-Veiga et al., 2009; Sloman et al., 2001), floods (Jensen and Johnsen, 1999; Pottinger et al., 2011), flow increase (Hackenberger et al., 2015; Sloman et al., 2002), general features of flow variability (Kemp et al., 2006; Nislow et al., 2004; Teichert et al., 2010) and turbulence (Enders et al., 2005, 2003; Sneddon et al., 2006) as the principle flow features of interest.

The effects of these flow features were addressed by analysing changes in whole-animal performance characteristics, namely growth (Harvey et al., 2006; Jensen and Johnsen, 1999; Kemp et al., 2006; Nislow et al., 2004), lipid content (Kemp et al., 2006), behaviour (Kemp et al., 2006; Sloman et al., 2001; Sneddon et al., 2006; Sunardi et al., 2007, 2005), body condition (Balcombe et al., 2012), survival (Harvey et al., 2006; Jensen and Johnsen, 1999; Nadeau et al., 2010), and disease incidence (Hockley et al., 2014; Maceda-Veiga et al., 2009). Assessing primary and secondary responses to stress was rarer, with corticosteroid concentrations (Pottinger et al., 2011; Sloman et al., 2001), metabolic changes (Nadeau et al. 2010; Pottinger et al. 2011) and changes in the cardio-respiratory system (Enders et al., 2005, 2003) as the main indicators of stress. The use of biomarkers as secondary responses was also used in biomonitoring programs to assess pollutant dissemination in streams with different hydrological regimes (Hackenberger et al., 2015).

Droughts have been reported to reduce fish condition, particularly when drought conditions were prolonged and when recent flow conditions were absent (Balcombe et al. 2012), and to increase disease incidence due to favourable conditions for parasitic multiplication (low water level combined with suitable water temperatures; Maceda-Veiga et al. 2009). Salmonids grew less when experiencing reduced mean summer discharges in field experiments (Nislow et al. 2004; Harvey et al. 2006) and when subjected to decreasing discharges and high fish densities in artificial streams mimicking seasonal flow variability (Teichert et al. 2010). Simulated drought conditions resulted in significant changes in the stability of the dominance hierarchies of salmonids (Sloman et al. 2001) and poecilids (Sneddon et al. 2006), suggesting that hierarchical structure was stronger in undisturbed flow conditions. When integrating whole-animal performance alterations with sub-organismal responses, the results were somewhat inconclusive; plasma cortisol levels were characteristic of unstressed fish (<10 ng/ml; Pankhurst 2011) and liver glycogen did not differ significantly in comparison to control conditions (Sloman et al. 2001).

Floods had diverse effects on the growth and survival of fish, varying with species and life stage (Jensen and Johnsen, 1999). Simulated high flow conditions resulted in decreased growth and poorer condition of cyprinids (Sunardi et al. 2007). However, when combining growth rate and lipid composition as responses to high flow conditions, juvenile salmonids either grew slower with no apparent effects on lipid composition or experienced lower lipid composition (higher energetic costs) with no apparent effect on growth (Kemp et al. 2006). These studies suggest that using solely growth (body weight measurements on a temporal scale) or body condition as responses to simulated high flow conditions might generate biased assumptions (Kemp et al. 2006). For example, when combining whole-animal performance changes (social behaviour) with sub-organismal responses (cortisol), researchers found that the hierarchical structure of salmonids exposed to spates in experimental tanks was disrupted (Sloman et al. 2002). The plasma cortisol levels were consistent with these findings and were significantly higher in comparison to the control group (Sloman et al. 2002). In another experiment,

salmonids were exposed in their final migration stage to fast velocity treatments which resulted in impaired physiological condition, shown by increased energetic costs (Nadeau et al. 2010).

Studies that used an integrated approach by assessing both whole animal changes (tertiary responses to stress) and sub-organismal indicators (primary and secondary responses to stress) highlighted the importance of adding diverse indicators of stress, other than growth or body condition, to assess fish performance under flow variability (Kemp et al., 2006; Sloman et al., 2002, 2001). Not only is it relevant to add diverse indicators of the stress-axis, but also to include different components of flow, for example, turbulence. Turbulent conditions are common in rivers inhabited by salmonids which prefer fast-flowing waters, and when used to estimate their activity metabolism, turbulence negatively affected swimming costs (Enders et al., 2005, 2003).

Not all organism level responses indicate causality between the stressor (flow variability) and the response under investigation. For example, Sloman et al. (2001) assessed the three types of responses to stress (primary, secondary and tertiary responses), finding no effect of the environmental perturbation on primary and secondary stress responses. A possible explanation was the timing that the blood samples were taken, which might have been inadequate to demonstrate a stress response. However, manipulating fish for blood removal more than one time during the same experiment could present an additional stressor that could confound conclusions about the stress response (Sloman et al. 2001).

Extreme hydrological regimes have been investigated as mechanisms of pollutant dissemination (Hackenberger et al., 2015; Pottinger et al., 2011). Although hydrological regime fluctuations were an additional stressor to fish, indicated by the significant variations in cortisol and glucose levels, the explaining mechanisms remained unclear (Pottinger et al. 2011). Interestingly, fish inhabiting fast-flowing waters affected by pollution were more susceptible to incurring increased stress as it was demonstrated by an increase in oxidative stress and DNA damage (Aniagu et al., 2006; Balcombe et al., 2012; Hackenberger et al., 2015). In respirometer experiments where fish were subjected to increasing water velocities, the higher swimming activity resulted in higher oxygen consumption rates (e.g. Enders et al. 2005; 2003). Higher fish swimming activity may result in an enhanced antioxidant potential (increasing enzymatic and non-enzymatic processes) (López-Cruz et al., 2010), thus if a riverine system is affected by pollution and unpredictable fluctuating or pulsed flows, the fish susceptibility to incur in oxidative stress has the potential to increase.

Most of these aforementioned studies examined extreme flow features, some of which may only be comparable to catastrophic floods or droughts in natural systems. Natural flow disturbances like the more frequent floods and droughts are intrinsic components of pristine fluvial ecosystems exerting an adaptive pressure over organisms (Lytle and Poff, 2004). These events are often critical for life-history stages, such as migration and reproduction, and although they are demanding they are not necessarily

stressful (Pankhurst 2011). In fact, eliminating these natural disturbances has potential stressful effects on fish by affecting life-cycle events, such as reproduction or growth (Balcombe et al., 2012; Lytle and Poff, 2004).

It is certain that different rates of natural disturbances result in increased primary and secondary responses and if they become chronic they can cause adverse effects on growth, disease resistance and behaviour. However, to identify the relationship between natural disturbances and the resultant stress response in the natural environment is still a challenge (Pottinger et al., 2011), enhanced by the effects of confounding environmental variables that cannot be controlled (Harvey et al. 2006; Pankhurst 2011) and may compromise the feasibility of the whole experiment design.

2.1.6 Responses in modified flow conditions

A considerable amount of scientific effort has been directed to evaluate the effects of modified flow regimes in riverine fish (Murchie et al., 2008; Souchon et al., 2008). Still, the organism level mechanisms explaining the effects of flow modifications in fluvial fish are poorly understood (Taylor et al., 2012).

The effects of modified flow regime were investigated in 26 of the 58 articles collected. Flow modifications associated with dams were of primary concern, either to address the effects of pulsed flows on swimming activity (S.ACocherell et al., 2011; Geist et al., 2005; Taylor et al., 2012), growth (Finch et al., 2015; Korman and Campana, 2009), survival (Fisk et al., 2013; Korman et al., 2011) and endocrine processes (Flodmark et al., 2002; Taylor et al., 2012), or the effects of fishway passage efficiency on migration (Burnett et al., 2014; Cocherell et al., 2011a; Pon et al., 2009a).

Whole-animal changes were the most common stress indicators while investigating the effects of modified flows in fish. For example, higher egg incubation mortalities were reported during fluctuating flows in large regulated rivers (Korman et al. 2011), in dewatering experiments performed in the natural environment (Casas-Mulet et al., 2015) and in experimentally controlled conditions (Fisk II et al. 2013). Growth was also negatively affected by hydropeaking conditions in YOY salmonids in large regulated rivers (Korman and Campana, 2009) and in juvenile salmonids in controlled experiments under stable low-flow and constant flow fluctuations (Flodmark et al., 2004). Some contradictory results have been reported for salmonids under simulated hydropeaking conditions. For example, growth and foraging were optimized in stable high flows in fiberglass tanks (Flodmark et al., 2004) while in an artificial stream no effects on growth or aggression were found (Flodmark et al., 2006). Surprisingly, in an adaptive management program at a dam on the Colorado River, juvenile cyprinid growth was lower in more natural steady flows in comparison to hydropeaking flows (Finch et al., 2015). However, purposed controlled floods resulted in higher chances of survival and growth in YOY salmonids (Korman et al. 2011), highlighting the interspecific differences in responses to flow. Although these studies reinforce

the need to set hydropower plant production operational measures to meet fish species flow requirements, the approximation of modified flow regimes to natural regimes does not always result in improved fish performance and the objectives of flow regulation should be carefully considered prior to development and implementation of a flow regime strategy.

The effects of pulsed flows as triggers of primary and secondary stress responses in fish were firstly investigated in an artificial stream by Flodmark et al. (2002). In this study, the velocity ranges and flow depths were characterized in detail, minimizing the effects of confounding environmental variables and allowing causality inferences between water velocities and stress responses. Interestingly, the highest plasma cortisol levels in brown trout (*Salmo trutta*) were reported for the short duration down ramping events (2 h), suggesting that the fish were experiencing an acute stress, but after six and twelve hours, plasma cortisol returned to pre-exposure levels (Flodmark et al. 2002). In the long-term seven-day experiment, after four days of flow fluctuations, no plasma cortisol response was observed, suggesting habituation to the stressor (Flodmark et al. 2002). The time of exposure to stressors has different implications on fish, ranging from habituation to compensation or exhaustion (Flodmark et al. 2002). Compensation occurs when after a repeated exposure to the flow fluctuation the fish adjusts physiologically or behaviourally (e.g. velocity refuging); exhaustion occurs when the stress-axis has been stimulated to a degree where a down-regulation of the system occurs and the fish are unable to react to the flow fluctuation (Schreck, 2000). In some cases when animals are exposed repeatedly to a stressful stimulus, stress responses are attenuated and the stimulus ceases to be considered a stressor (Schreck 2000).

Flodmark et al. (2002) reported no changes in blood glucose levels, suggesting that using solely blood glucose as a stress indicator might mask other responses and lead to inaccurate assumptions. This study could be strengthened by integrating quantitative behaviour metrics that would support behavioural assumptions according to the cortisol results. For example, in a study integrating behaviour and physiological telemetry (electromyogram (EMG) activity), the discharge duration, frequency and magnitude had a significant positive effect on the swimming activity and cortisol levels of a salmonid in a hydropeaking reach (Taylor et al. 2012). Although there was a significant increase in cortisol concentrations, they corresponded to typical values of unstressed fish (<10 ng/ml; Pankhurst 2011) and lactate loads were not indicative of fish swimming anaerobically. In addition, there was a considerable amount of variance that remained unexplained, possibly due to other environmental variables or inter and intra-specific variables that could not be controlled. In a controlled experiment simulating dewatering during down ramping, a salmonid moved ~ 2.5 times more in comparison to the reference channel although blood cortisol levels, blood glucose and energy stores did not persist over time after the stimulus (Krimmer et al., 2011). Short-term elevated levels of cortisol have also been reported in laboratory dewatering experiments but were also followed by rapid habituation (Arnekleiv et al., 2004). These findings suggest that either the flow regime imposed was not stressful or the target species

became habituated to the disturbance (Flodmark et al. 2002; Arnekleiv et al. 2004). Either way, knowing the flow thresholds (magnitude, duration, frequency and rate of change) that might produce a stress response in fish is difficult. In addition, the water level changes that occur during the dewatering stage or the flooding that occurs during the pulsed flow cycles might result in contradictory conclusions; increased stress during dewatering (e.g. Flodmark et al. 2002) and small home ranges coupled with lower swimming costs during flooding (e.g. Cocherell et al., 2011b). However, it is necessary to find out whether flow variability represents an actual stress and if it does, to use bioindicators that effectively quantify the stress response and to define effective flow variability mitigation strategies. High flows may characterize fishway entry and passage and are usually associated with anaerobic conditions, increasing the susceptibility of migratory fish to stress (Burnett et al. 2014). Additionally, when moving through some fishways at large dams, fish may be subjected to fatigue-recovery cycles (Cai et al., 2015, 2014; Pon et al., 2009b). Although the anaerobic conditions associated with high attraction flows explained in part the higher mortalities of salmonids after passage success (Burnett et al. 2014), physiological indicators (plasma cortisol, lactate and glucose, hematocrit, and ionic status) were not indicative of stress or exhaustive exercise within the operational range studied for the same fish in the same dam (Pon et al., 2009a). In sturgeon, there was a significant increase in corticosteroid levels (plasma cortisol) and metabolic changes (haematocrit, pH, osmolality and lactate) in an experimental fishway, but 24 hours post-experiment, indicator values returned to pre-stress levels (Cocherell et al., 2011a). Apparently, to efficiently enter and surpass these obstacles, fish have to negotiate under anaerobic conditions and, depending on flow intensity and individual characteristics (e.g. life stage, condition) the responses to stress might be different and sometimes contradictory.

Physiological telemetry (e.g. EMG activity) was a common (7 of 26) tool used to estimate the energetic costs associated with exposure to daily pulsed flows (estimate aerobic swimming-muscle activity of free-swimming fish). These high flow fluctuations were indicated to increase the energetic costs of mountain whitefish (*Prosopium williamsoni*; Taylor et al. 2012), rainbow trout (*Oncorhynchus mykiss*; Cocherell et al., 2011b) and white sturgeon (*Acipenser transmontanus*; Geist et al. 2005) at different discharge magnitudes and rates of change (Table 2.2). When calibrated to swimming speed (respirometry), these studies demonstrated that oxygen consumption increased with swimming speed. In opposition to Taylor et al. (2012), there was no evident flow-stress response (Cocherell et al., 2011b; Geist et al., 2005) and overall free-swimming fish speed was lower than their individual critical swimming speeds (Cocherell et al., 2011b), with no indication of exhaustive exercise. Apparently, above a certain threshold there was a decrease in swimming speed (Cocherell et al., 2011b) and EMG results revealed a small home range where movements were restricted by high flows with minimal longitudinal movements during the increased pulsed flows (Cocherell et al., 2011b; Geist et al., 2005). The small home range during the pulses was explained either by the higher habitat availability caused by inundation (Cocherell et al., 2011b) or the duration of the low-flow period, reducing the time required to search for new habitats (Geist et al. 2005). The reluctance to change lateral position in response to sudden increases in flow has

been demonstrated in experimental studies performed with juvenile fish (Kemp et al., 2003; Vehanen et al., 2000; Vilizzi and Copp, 2005), suggesting that in these highly modified environments fish species perform restricted movements.

All these studies highlight the importance of integrating tools, for example swimming activity with physiological indicators, to effectively evaluate modified flows at the organism level. However, the limitations related to experimental design, namely study location or experiment type (natural or manipulated laboratory flow conditions) are known and have to be considered. In field experiments, external factors and biotic and abiotic interactions occur and cannot be controlled, whereas in laboratory conditions fish might not exhibit their full genetic potential. In natural conditions, the difficulty in observing fish behaviour individually inevitably leads to assumptions that have not been experimentally verified, such as compensation behaviour (searching for 'flow refuging') during high flows and foraging or social interactions during low flows. Although laboratory studies have the advantage to eliminate other confounding natural or anthropogenic factors, confinement does not provide optimal conditions to express whole-fish performance. Furthermore, fish swimming abilities can vary widely for a single species or a group of species which demonstrate performance similarity, such as subcarangiform or anguilliform swimmers (Katopodis and Gervais, 2016). The different fish morphologies and swimming hydrodynamics result in diverse swimming performances and consequently in energetic discrepancies (Pettersson and Hedenstrom, 2000; Sfakiotakis et al., 1999). These additional constraints in both laboratory and natural conditions might justify the low number of articles that focus on organism level responses to flow variability. Certainly, integrated approaches that measure both whole-animal performance and physiological responses provide more insight (Taylor et al. 2012). The resultant findings would provide useful guidelines for water managers to define flow requirements according to species and life-stage, propose hydropower plant operational measures or to design effective fishways.

2.1.7 Biomarkers for flow variability

Environmental physiologists have been struggling to understand and quantify the effects of flow variability on fish physiology. However, it is essential to evaluate the validity of the physiological responses as adequate indicators of a flow disturbance and whether or not they can be used as reference values to address the potential of a flow disturbance to cause a stress response in fish. The value of stress physiology as a tool to evaluate the impact of natural or anthropogenic disturbances on a specimen is well known (Pankhurst 2011); additionally, fish are highly susceptible to disturbance (Poff and Zimmerman, 2010), thus they can be used as potential sentinel organisms to quantify the extent to which a certain flow disturbance can cause a physiological response (Pottinger et al. 2011). Not surprisingly, salmonids were the most studied group (32 of 58) and were mainly studied to assess the effects of modified flow regimes (17 of 26). Growth was the most studied indicator of whole-animal

performance to changes in flow regimes, whether they were natural (7 of 18) or modified (6 of 26) flows. Yet, relatively few studies determined if there was a stress response to a defined flow variability stimulus (but see Table 2.2). Short-term responses to stress were most commonly quantified by determining plasma cortisol concentration, as a primary response, and swimming costs, measured by oxygen consumption or blood lactate, as a secondary response (Table 2.2). Cortisol concentration was directly associated with the studied threshold of flow variability, and increasing swimming costs, measured by oxygen consumption, were associated with fast water velocities (Table 2.2). Hematocrit and osmolality were negatively linked with the flow stimulus and plasma glucose and lactate concentrations were more variable and difficult to interpret (Table 2.2). Although there is enough evidence that basal cortisol levels in wild teleosts are usually <10ng/ml (Pankhurst 2011) or even <5 ng/ml (Pickering and Pottinger, 1989), what these baseline values represent when considering the environmental factor 'flow' has been scarcely assessed. Additionally, translating these values to thresholds according to environmental flow requirements is unknown. The main constraints to identify these thresholds reside in inherent characteristics of the fluvial ecosystem and the species in study and in the sampling procedure (fish handling and time interval between flow disturbance exposure and sample collection). Apparently there is no method that eliminates sampling stress to estimate short-term primary and secondary responses to stress (Arnekleiv et al., 2004; Pankhurst, 2011). To minimize the effect of handling and external confounding factors, authors have suggested the use of calibration curves by conducting blood sampling for analysis only once at the end of the experiment, in experimental and reference fish, thus avoiding fish behaviour disturbance throughout the analysis (Sloman et al. 2001; Krimmer et al. 2011). Establishing credible resting or baseline data for unstressed fish in the wild is difficult but of critical importance to understand how the same endocrine variables might change in relation to processes where the primary driver may not be the response to a stressor (Pankhurst 2011).

Studies analysing fish swimming activity demonstrated that increases in water velocity resulted in higher energetic costs (Table 2.2), particularly when combined with other environmental factors such as temperature or predation (Enders et al., 2005, 2003; Gregory and Wood, 1998; Nadeau et al., 2010).Considering the definition of energetic cost, i.e. the necessary energy to transport one body mass by one unit distance (Schmidt-Nielsen, 1972), and fish inter an intra-specific characteristics (e.g. morphology, Fu et al. 2014, 2013; predation, Sloman et al. 2001), it makes sense that increasing water velocities results in higher oxygen consumption rates and that these changes could represent a transient stress response. The challenge is in finding what the minimum cost of transport given a determined water velocity, i.e. the optimal swimming speed (U_{opt}), and the maximum cost of transport given the maximum velocity that the fish can sustain, i.e. the critical swimming speed (U_{crit}).

Table 2.2 Studies that analysed a specific flow feature and quantified a stress response. Flow features were separated according to natural or modified flow regime. Studies performed under controlled conditions simulated natural and/or modified flows. Flow features and stress indicator thresholds are indicated. (I), (II), (III) - Primary, Secondary and Tertiary stress-axis responses, respectively. (s.d.) Standard deviation; (Hct) Hematocrit; (SMR) Standard metabolic rate; (MEL) Metabolic Energy Loss; (GSH) Glutathione; (SOD) superoxide dismutase;(HSI) Hepatosomatic index; (TBF) Tail beat frequency.

A: Natural						
Flow feature	**Author**	**Taxa**	**Experiment type**	**Flow feature threshold**	**Measured responses to stress**	**Indication of stress**
Spates	Sloman et al. 2002	Salmonidae	Experimental flume	Water velocity: 0.84±0.05 m/s	(I)Plasma cortisol; (II)HSI; (III)Growth	Plasma cortisol: ↑25.75±8.70 ng/ml HSI ↓ 0.87±0.04
Flow increase	Sunardi et al. 2007	Cyprinidae	Tank	Riffle 0.11±0.072 m/s;Pool 0.02±0.037 m/s	(II)SMR,MEL; (III)Growth, Conditon	↑ Energetic costs
Drought	Sloman et al. 2001	Salmonidae	Tank	↓ 37% (Water level)	(I)Plasma cortisol; (II)Gill epithelial chloride cell density, Interrenal nuclear areas; (III)Growth	Absence of dominance hierarchy
	Harvey et al. 2006	Salmonidae	Artificial stream	↓ 75-80% (Water level)	(III)Growth	↓ 8.5 fold
Low/High	Nadeau et al. 2010	Salmonidae	Experimental raceways	Fast velocity: 0.38-0.39 m/s Slow velocity: 0.10-0.12 m/s	(II)Plasma osmolality, Lactate, Glucose, Hct, Chloride, Potassium, Sodium, Gill ATPase, GSE, Concentration of lipid water, protein, ash	↑ Lactate and Glucose; ↓ GSE Hct Osmolality; Chloride; Sodium
Turbulence	Enders et al. 2003	Salmonidae	Respirometer	Water velocity: Mean 0.18, 0.23 m/s; s.d. 0.05, 0.08 m/s	(II)Swimming costs (Oxygen metabolism)	Swimming costs: ↑ 1.3-1.6 fold Oxygen Consumption: 1.46-4.42 mg O_2/h (1.4 to 4.3 times SMR)
	Enders et al. 2005	Salmonidae		Water velocity: Mean 0.18, 0.23 and 0.40 m/s; s.d. 0.05,0.08 and 0.10 m/s	(II)Swimming costs (Oxygen metabolism)	Swimming costs: ↑ 9.3 fold
B:Modified						
Pulsed flows	Krimmer et al. 2011	Salmonidae	Artificial stream	↓ 50-75% in 4h	(I)Plasma cortisol; (II) Plasma glucose, Bioenergetics; (III) Growth	↑ 2.5 fold of small scale movement; ↓ body mass condition; No indication of stress according to (I) and (II)
	Cocherell et al. 2011b	Salmonidae	River/Respirometer	5-35 m^3/s(weekdays); 5- >45 m^3/s (weekends)	(II) Swimming costs (Oxygen consumption)	> 44 m^3/s: ↓ swimming speed and activity
	Flodmark et al. 2002	Salmonidae	Artificial stream	Discharge – Water velocity 190 dm^3/s - 0.26-0.33 m/s; 40 dm^3/s - 0.09-0.13 m/s	(I) Blood cortisol; (II) Blood glucose	Blood cortisol:59.4±35.3 ng/ml (2h-down ramping); 61.3±26.8 ng/ml (1 day-daily cyclical fluctuations)
	Geist et al. 2005	Acipenseridae	River/Respirometer	Pulsed 192–836 m^3/s; High 442–629 m^3/s; Low 275–284 m^3/s	(II)Swimming costs (Oxygen consumption)	↑ Oxygen consumption: 140.2-306.5 mg O_2 /kg/h (attributed to water temperature and light levels)
	Taylor et al. 2012	Salmonidae	River	Hourly discharge: Mean: 621 m^3/s; Range:0–1770 m^3/s	(I) Plasma cortisol; (II) Blood lactate	Plasma cortisol: ↑ 1.60 ± 0.09 ng/ml
Flow increase	Aniagu et al. 2006	Cyprinidae	Experimental flume	Water velocity: 0.2 m/s every 15 min	(II)DNA damage; (II)Antioxidant status: GSH and SOD	DNA damage: 25% tail DNA in exercised fish; DNA strand breaks: % 22 in exercised gills; oxidative stress
Drought	Arnekleiv et al. 2004	Salmonidae	Artificial stream	Water velocity: 0-0.64 m/s; Water Depth: 10-50 cm	(I)Plasma cortisol; (II)Blood glucose	No stress indication to dewatering
Fishway	Burnett et al. 2014	Salmonidae	Fishway passage	Discharge: 1.2±2.0 x 10^{-3} m^3/s	(II) Oxygen consumption	↑ Anaerobic metabolism
	Cocherell et al. 2011a	Acipenseridae		Water velocity: 1.7–2.1 m/s; Head tank discharge: 0.88 m^3/s	(I)Plasma cortisol; (II)Hct, plasma pH Osmolality, Chloride, Ion concentration; Lactate,Glucose	↑Plasma cortisol: 78.5±14.5 ng/ml Lactate: 5.77 ±2.11 ng/ml Hct: 28.0±1.5%; ↓ pH:7.75±0.02 post swimming (24h)
	Pon et al., 2009b	Salmonidae		Discharge: Intermediate -12.7 m^3/s High - 15.8 m^3/s; Low - 11.0 m^3/s	(I)Plasma cortisol (II)Plasma lactate, Glucose Ions, Osmolality; Hct	No indication of stress or exhaustive exercise
Low/High	Young et al. 2010	Osmeridae	Fish screen	Approach: 0; 0.06; 0.10; 0.15 m/s; Sweeping: 0; 0.31; 0.62; m/s	(I)Plasma cortisol; (II) Hct, pH	↑ Plasma cortisol: ↑Approach velocities and ↑ Sweeping velocities - range ~ 150-500 ng/ml

Studies using respirometers, where swimming speeds were a proxy for energy expenditure, or exercise training, where water velocity was a proxy for fitness optimization, also demonstrated that water velocity increments resulted, respectively, in higher swimming costs (Fu et al., 2015; Gregory and Wood, 1998), but also in improved fitness (Woodward and Smith, 1985; Young and Cech, 1993; Young and Cech Jr, 1994a, 1994b). These studies analysed the swimming costs associated with increases in water velocity (respirometers) or in fitness improvement by confinement in constant flow conditions (exercise training) and demonstrated that increasing water velocities resulted in sub-organismal responses (changes in plasma cortisol, glucose, lactate) and whole-animal performance changes (growth, swimming behaviour) that could compromise the full expression of the fish genetic potential. The intensity of the manipulated stimulus (duration, frequency, magnitude, rate of change) together with individual fish fitness were indicated to be the main factors influencing fish whole-animal performance and endocrine responses that in the natural environment would be translated in diversified swimming behaviours such as habituation, compensation and/or exhaustion (Flodmark et al. 2002). Using these experimental approaches to simulate flow variability conditions that fish would encounter in the natural environment, would provide valuable insight on the energetic costs associated to both optimal and critical swimming performances. This resultant knowledge can be applied for the design of mitigation measures that maximize habitat heterogeneity and optimize the fish chances of seeking velocity shelters and widening the lateral and longitudinal range of movements by increasing habitat connectivity which is particularly important during the up- and down-ramping in highly regulated rivers.

2.1.8 Conclusions

Scientists and water managers worldwide have been struggling to define effective water management guidelines that optimize environmental flow regimes, thus increasing the sustainability and ecological integrity of the fluvial system (e.g. Jones, 2014; Katopodis, 2005; Souchon et al., 2008). At an individual scale, scientific effort has been dedicated to identify fish flow requirements at critical life stages, such as rearing or migrating to spawn and forage, with particular emphasis given to salmonid species (Enders et al., 2009; Fenkes et al., 2016; Signore et al., 2016), or to swimming behaviour (Liao, 2007).

Fluvial fish are evolved to live in environments with variable flow but are adapted for certain flow conditions. Flow variation can therefore represent a potential stressor for fish in conditions beyond their optima. This review summarized the growing body of research reporting the impacts of flow variability at an organism level. Natural and anthropogenic flow variability presents a potential stressor to fish,

demonstrated by the changes in physiological processes and/or whole-animal performance characteristics. Increased levels of blood cortisol (primary response), changes in oxygen metabolism and increased levels of blood glucose and lactate (secondary responses) were some of the most relevant indications that changes in flow components contribute to a transient short-duration stress. These results were more evident under pulsed flow scenarios (e.g. Flodmark et al. 2002) and in the highly unstable flow conditions occurring in fishway entry and passage (e.g. Cocherell et al., 2011a) where the cortisol levels increased 6-8-fold in comparison to the reference value for unstressed fish. These endocrine changes may be followed by habituation or compensation, indicating that fish trigger adaptive mechanisms aiming at re-establishing homeostasis. When the organism can no longer maintain homeostasis, critical life-stages such as reproduction, foraging, escaping from predators or migrating, can be compromised. For example, under hydropeaking scenarios the larval survival was negatively affected (Casas-Mulet et al. 2015). Thus, under severe flow conditions the energetic cost associated to swimming activity increases (e.g. Geist et al. 2005; Sunardi et al. 2007) and less energy will be directed to those critical processes.

Species-specific flow ranges have been defined from scientific assessments, particularly for salmonid species given dewatering conditions (Harvey et al., 2006; Krimmer et al., 2011; Sloman et al., 2001) and velocities (e.g. 0.35 m/s; Flodmark et al. 2002, Nadeau et al. 2010). However, specific flow components, such as duration, magnitude, frequency and rate of change, together with external biotic and abiotic factors are still strong limitations to find flow thresholds that present real stressors to fish. What remains unclear is the extent to which changes in those flow components trigger a stress response or allow habituation and compensation (e.g. Flodmark et al. 2002) to avoid long-term effects.

2.1.9 Future research directions

There is a clear imbalance in the number of studies examining whole-animal performance and sub-organismal stress responses to flow variability, favouring the first, although there is a diverse set of physiological indicators and measuring tools that can be integrated for such studies. The lack of research focusing on short-term stress-axis responses might be attributable to the difficulty in establishing a direct association between the flow component affecting the fish and the indicator response. According to the studies conducted so far, the reported reasons explaining this reluctance include the time it takes between stimulus duration and sample collection, the external environmental factors that cannot be controlled and the facilities where the experiment occurs (field or laboratory). When addressing the effects of flow variability at an organism level, sentinel species should be proposed not only as indicators of whether or not flow presents a stressor but by exposing them to increasing flow stimulus

and determine the threshold that results in a potential response. These species could also be used to monitor mitigation measures and perform adaptive management (Adams and Ham, 2011). Additionally, studies that bridge the gap between individual responses (whole and sub-organismal) and populations, communities and the fluvial ecosystem are necessary (e.g. Harvey et al., 2006; Sloman et al., 2002, 2001). Considering the role of flow regime as a driver of fish life-history adaptations, future research should be directed in finding robust biomarkers that not only link the effects of flow disturbances at the individual level to populations but that could also be included in conservation and environmental management policy (Cooke and O'Connor, 2010).

According to the diverse physiological tools available to quantify how flow variability affects fish behaviour, it is difficult to choose the most reliable and robust for assessing the potential for a stress response. Because there already exists an accepted concentration for plasma cortisol as an indicator of unstressed teleost fish, this was the elected short-term stress response and it was used in 16 of the 58 collected articles. To establish valid baseline values for unstressed wild fish associated either to natural flow dynamics (Krimmer et al., 2011; Sloman et al., 2001), or according to the fluvial ecosystem that they inhabit, it is essential to have a comparable reference value, as long as the sampling techniques are minimally invasive to reduce handling stress, and external factors that otherwise could not be explained are eliminated (Arnekleiv et al. 2004, Finch et al. 2015).

It is essential to integrate effective measuring tools to quantify the effects of flow variability and adequate indicators of a stress response, to avoid inaccurate assumptions that would otherwise result in the implementation of costly mitigation measures that would not improve the fluvial system. For example, changing operation procedures in hydropower production plants to achieve a more natural flow regime did not result in increased fish growth and revealed to be counterproductive (Finch et al. 2015). In this study, growth was the only used functional indicator and it could be supported by the use of other fish fitness indicators associated, for example, to swimming costs (e.g. lipid metabolism, oxygen consumption) and even endocrine indicators of stress (plasma cortisol) or exhaustive exercise (plasma lactate). Physiological telemetry is a promising tool to address how flow disturbances induce organism level responses in the wild (Cocherell et al., 2011b; Hasler et al., 2014, 2012, Taylor et al., 2014, 2013, 2012). Taylor et al. (2012) combined EMG responses with primary stress responses and although the results on whether the pulsed flows were contributing to a stress response were not conclusive, quantifying the available types of stress responses prevented inaccurate assumptions that could otherwise result in the implementation of unnecessary mitigation measures. Using behavioural metrics supported by direct observation or using small scale movement tracking technologies (accelerometry) could also be alternatives to provide evidence on how flow affects individual-level performance. These

are particularly relevant, as the fast changes occurring downstream of a hydropower plant seem to induce only small scale movements in fish (Krimmer et al. 2011). The combination of acceleration models with oxygen metabolism has demonstrated how the extreme flow conditions which are sometimes associated with fishway entrance and passage can be stressful to fluvial fish performing spawning migrations (Burnett et al. 2014; Table 2). Understanding the effects on movement patterns and small scale habitat preferences seems to be an important research focus aiming at promoting effective flow management in these highly fluctuating environments. Finally, these could be added to powerful statistical modelling to make predictions of future scenarios (e.g. Cocherell et al., 2011a,b; Taylor et al., 2013).

Other aspects of flow variability research requiring further attention include how flow behaves according to the interface of the fish body and fins (Liao 2007) and how the body shape correlates with swimming performance and other ecological parameters (Fu et al., 2013; Katopodis and Gervais, 2016). Although this line of investigation does not address flow variability as a potential stressor in isolation, it increases the awareness of the selective pressure that flow variability can exert on fish phenotype and increase the individual potential to benefit from flow variability to reduce the swimming costs associated with the increasingly higher fluctuating flow environments (Fu et al. 2013). Using experimental protocols that are able to quantify endocrine responses without handling stress (Zelnik & Goldspink, 1981), combine them with protocols to analyse fish swimming energetics (e.g. Fu et al., 2015, 2014; Taguchi and Liao, 2011) and experimental studies that simulate flow variability conditions that fish would encounter in the natural environment (e.g. Fu et al., 2015, 2014, Sloman et al., 2002, 2001) could be a valuable experimental approach that would explain if and how those flow constraints do actually represent a stressor to fish and to understand the adaptive mechanisms to flow variability.

Establishing experimental facilities in the fluvial system, either by installing cages (Hackenberger et al. 2015) or creating artificial streams (Casas-Mulet et al. 2015) to investigate both natural and highly modified flow conditions was effective in demonstrating the negative effects of pulsed events in the physiological condition of adult fish and in hatching success and larval survival, respectively. By using experimental facilities in the wild it was possible to isolate environmental variables, eliminate the challenges of confinement and to establish an association between flow variability and an organism level stress response. Notably, even in confinement, fluvial fish were capable of expressing swimming behaviour identical to natural conditions, (e.g. dominance hierarchies, Sloman et al. 2002, 2001; foraging, Flodmark et al. 2004) and responses to stress typical of unstressed wild fish have been reported. However, as confinement hinders the capacity of fish to fully express its performance potential, the possibility of performing experiments in the natural environment should always be

considered during the critical phase of experimental design (Patterson et al. 2004). There is certainly a need for large ecohydraulic experimental flumes or stream channels where flow can be manipulated over relevant timescales. Knowing that these experiments aim at answering to local and regional scale problems related to flow modifications (e.g. Casas-Mulet et al., 2015; Taylor et al., 2012), their outcome can function as groundwork to assess flow modification problems at the river catchment scale.

Although this review emphasizes the difficulty in isolating environmental variables when analysing stress responses to flow (Harvey et al., 2006; Krimmer et al., 2011; Sloman et al., 2001), and thus in determining the real basis for the physiological response, field experiments conducted in natural or highly modified flow demonstrated that extreme flow conditions (e.g. floods, droughts, dewatering and pulsed flows) presented potential stressors to fluvial fish.

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2.1.11 References

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3

Responses of Iberian barbel to simulated hydropeaking: the role of lateral refuges

"Every one of us matters and has a role to play. Every one of us makes an impact on the world around us – every day. And we can choose what sort of impact we can make."

Jane Goodall

3.1 Do artificial velocity refuges mitigate the physiological and behavioural consequences of hydropeaking on a freshwater Iberian cyprinid?

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3.1.1 Abstract

The rapid flow fluctuations experienced downstream of hydropeaking facilities can alter the river hydromorphology. Given the dependence of riverine fish on physical habitat, those alterations have the potential to change the physiology and behaviour of fish. We assessed whether artificial velocity refuges mitigated the physiological and behavioural consequences of hydropeaking for the Iberian barbel (Luciobarbus bocagei). Hydropeaking trials were conducted in an indoor flume equipped with deflectors that created low flow velocity areas to serve as refuges. The FLOW-3D® was used to obtain detailed characterizations of the different velocity fields, which facilitated the interpretation of fish responses. Changes in flow magnitude and duration triggered stress responses, demonstrated by the increased blood glucose levels in the single up-ramping event for 60 $l.s^{-1}$ and in the step up-ramping event. Fish tended to seek out velocity refuges to avoid higher flow velocities and harsh hydraulic conditions at peak-flows, and during the longer events. The movement behaviour frequency increased when fish were subjected to the highest peak-flow (60 $l.s^{-1}$), particularly the individual sprints, and the drifts. For the base-flow (7 $l.s^{-1}$) and the lowest peak-flow (20 $l.s^{-1}$) conditions, fish swam freely in the flume, whereas in the harshest hydraulic conditions they showed more difficulty in finding velocity refuges. This research presents a novel approach by combining physiology and behavioural observations with hydraulic modelling to assess the extent to which artificial flow refuges mitigate the consequences of hydropeaking. Our work serves as a model approach for future mitigation studies for fish in hydropeaking rivers.

Keywords

Hydropeaking; Pulsed flows; Physiology; Velocity refuges; Movement behaviour; Iberian barbel

3.1.2 Introduction

Flow regime is an intrinsic characteristic of freshwater ecosystems, defining their ecological function and natural dynamics (Poff et al., 1997). Aquatic species have evolved adaptive traits to cope with the inherent predictability of natural flow disturbances (Lytle and Poff, 2004; Pankhurst, 2011). However, the continuous disruption of natural flow regimes has drastically altered the river physical character (Dudgeon et al., 2006), affecting critical life-cycle events of freshwater biota. Given the increasing global demand for energy, it is expected that hydropower production will increase as a result of low production costs, relatively low carbon emissions, and high efficiency to respond to peak demand (Zarfl et al., 2015). The large and rapid flow fluctuations in response to the sub daily or daily changes in hydroelectricity demand, i.e. hydropeaking (Cushman, 1985; Young et al., 2011) have the potential to alter the ecological function in freshwater ecosystems.

There has been an increasing effort to determine the ecological impacts of hydropeaking on downstream fish populations and communities including mechanistic research to understand the basis for such changes at the individual level. For example, researchers have studied the effects of rapid changes in flow on metabolic, i.e. changes in blood physiology (Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012) and swimming costs (Cocherell et al., 2011; Geist et al., 2005), ontogenetic changes, i.e. changes in growth (Krimmer et al., 2011; Puffer et al., 2015), reproductive success (Burnett et al., 2014) or survival (Korman et al., 2011). Furthermore, movement behaviour is crucial to assure the success of life-cycle stages namely growth, survival and reproduction (Kahler et al., 2001). In hydropeaking rivers, downstream displacement (Boavida et al., 2017), smaller scale (Jones and Petreman, 2015; Krimmer et al., 2011; Taylor et al., 2014, 2013) to larger scale movements (De Vocht and Baras, 2003), reproductive migration changes (Burnett et al., 2014), and stranding due to dewatering (Saltveit et al., 2001; Young et al., 2011) are the most documented examples of consequences for fish. However, contradictory results have emerged while studying the effects of pulsed flows on fish movement behaviour; either there was a notable change (e.g. Boavida et al., 2017; Krimmer et al., 2011), or there was no indication of an altered movement behaviour (e.g. Flodmark et al., 2006; Jones and Petreman, 2015), or an inconsistent effect was found, possibly explained by the availability of velocity refuges that reduced the need to move (e.g. Scruton et al., 2005). These findings suggest that the movement behaviour of fish in rivers affected by hydropeaking strongly depends on river morphology. It would be expected that a heterogeneous river habitat would provide more velocity refuges (Person et al., 2014; Vehanen et al., 2000). However, higher density of boulders and blocks may increase hydraulic instability and stranding risk under hydropeaking conditions (Auer et al., 2017; Tuhtan

et al., 2012). This knowledge is necessary to understand which hydraulic conditions influence fish movements.

Fish responses to stress are considered an adaptive mechanism for fish to cope with any external perceived stressors and maintain homeostasis (Pankhurst, 2011). In cases where the stressor persists over time, deleterious effects on overall fish performance (escaping predators, finding refuge, foraging) and in specific life stages (reproduction, growth) will likely occur (Barton, 2002; Pankhurst, 2011). Although changes in the blood physiology have occurred in down-ramping conditions (Arnekleiv et al., 2004; Flodmark et al., 2002; Krimmer et al., 2011), the flow conditions which trigger the stress response and the extent to which their severity may cause long-term deleterious effects remain unknown. Studies conducted in the field or in laboratory conditions assert that the absence of a physiological response after a severe flow component fluctuation indicates that fish were seeking out velocity refuges (i.e. flow refuging), although this possibility has not been properly assessed (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012). In field conditions, there is an additional difficulty in explaining the large variance in the movement behaviour results and in sub-organismal responses (e.g. Taylor et al., 2013, 2012), which is not favourable to the design and implementation of effective mitigation measures. It is still difficult to isolate variables and find a mechanistic link between a rapid flow change and a measurable fish response (Costa et al., 2017). The identification of this cause-effect association is essential to determine whether there is a biologically-meaningful negative impact, and if it is indeed necessary to implement mitigation strategies.

Given this evidence, performing experiments under controlled environmental conditions has been encouraged (Young et al., 2011) as they reduce the uncertainty caused by confounding external variables. Specifically, by enabling visual observations, controlled lab experiments enable the interpretation of sub-organismal responses (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012) , and have been demonstrated to be a valid approach in understanding smaller scale behavioural and physiological changes (Auer et al., 2017; Flodmark et al., 2002; Ribi et al., 2014). However, very few studies evaluate fish responses to structural habitat mitigation measures (Ribi et al., 2014) and only a handful propose mitigation measures based on hydrodynamic models (e.g. Boavida et al., 2015; Person et al., 2014).

The effects of hydropeaking events have been studied mainly for the fast swimming salmonids (Arnekleiv et al., 2004; Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012). Conversely, the movement behaviour and habitat preferences of cyprinids under rapid flow changes have been scarcely studied (Vilizzi and Copp, 2005). Being more susceptible to downstream displacement due to their low

swimming ability (Taylor and Cooke, 2012) and as the most representative group of fish inhabiting Portuguese rivers, mainly consisting of autochthonous species, it seems important to understand the effects of rapid flow changes on cyprinids to encourage the design of successful structural mitigation measures. In the present study, young adults of Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864), abbreviated as *L. bocagei*, an endemic potamodromous cyprinid of the Iberian Peninsula, were selected. The fingerlings and juveniles are predominantly rheophilic (Martínez-Capel and García de Jalón, 1999), as well as the spawning adults, choosing faster currents to migrate upstream during reproductive season (Rodriguez-Ruiz et al., 1992). Outside this period, the adults of *L. bocagei* tend to be limnophilic, preferring lower velocities. We adopted an integrative approach to better understand the biological consequences of simulated hydropeaking conditions on *L. bocagei*, incorporating behavioural metrics, blood physiology and hydraulic modelling. The main objective of this study was to assess the effects of simulated hydropeaking conditions on the stress physiology and movement behaviour of *L. bocagei* in an experimental indoor flume equipped with lateral velocity refuges. Specifically, the following null hypotheses were tested: 1) flow magnitude and hydropeaking event duration do not cause significant changes in the levels of blood glucose and lactate of *L. bocagei*, nor in the movement behaviour of this species in the flume; 2) *L. bocagei* uses equally the available refuges and remaining flume area when subjected to peak-flows and base-flows.

3.1.3 Materials and methods

Fish sampling, transportation and acclimation

Fish sampling took place at the Lisandro River (38.900554º N, -9.365715º W), a medium-sized tributary of the Tagus River, central Portugal. This tributary is not influenced by hydropeaking which makes it a suitable source of fish that are naïve to hydropeaking events. Fish were sampled once a week during three non-consecutive weeks between October and November 2015 using a low-voltage (400 V) electrofishing gear (Hans Grassl IG-200), according to European norms (European Committee for Standardization – CEN (CEN, 2003) and national guidelines (INAG, 2008). In each sampling occasion no more than 80 fish were captured, resulting in a total of 120 fish (mean total length (TL) ± standard deviation (SD) cm; 18.0 ± 3.8 cm; mean total weight (TW) ± SD g; 56.3 ± 36.6 g). After electrofishing, fish were transported in a constantly aerated fish transport tank (Linn Thermoport 190 l) to the laboratory. The fishes were equally transferred to two 900 l tanks with ambient temperature and natural photoperiod for a 48-72 hours acclimation period where no food was provided. To eliminate the potential effects of acclimation time, the fishes that were subjected to different acclimation periods were also subjected to different flow events. Each tank was covered with a fine meshed garden net and

contained four clay roof tile refuges. Tank water was biologically filtered and permanently aerated. Water quality parameters (mean ± SD) were monitored in a daily basis using a multi parameter probe (YSI 556 MPS) for temperature (22.6 ± 1.6 ^{o}C), pH (7.84 ± 0.84), dissolved oxygen (7.62 ± 0.66 mg.l^{-1}) and conductivity (287.7 ± 60.2 µS.cm^{-1}), and in a weekly basis using photometry (WTW- Spectroflex 6600) for nitrites (0.08 ± 0.09 mg.l^{-1}), ammonia (0.002 ± 0.003 mg.l^{-1}), and chlorine (0.12 ± 0.12 mg.l^{-1}). Partial water changes (15%) were performed every other day. After the acclimation period fish were fed with a commercial diet for benthic species every day at night to avoid additional stress caused by food deprivation. The flume water temperature (24.4 ± 2.1 ^{o}C) and dissolved oxygen (7.00 ± 0.74 mg.l^{-1}) were monitored twice a day. The water quality parameters were in accordance with the national legislation for water quality standards to protect and improve the aquatic environment according to water use for cyprinid waters (Ministry of the Environment, 1998).

The experiments took place between the 5th of October and the 17th of November 2015 in an indoor artificial flume located at the Hydraulics and Environmental Laboratory (IST, University of Lisbon, Portugal) (Figure 3.1). The flume has a rectangular cross-section (8m long, 0.7m wide and 0.8m high), and was built on a steel frame with glass panels on both sides. An upstream reservoir controlled by a plane gate enabled rapid variation of flow and a downstream flap gate controlled the water level. The usable area for fish was limited by two perforated metallic panels creating a 6.5 m long-reach usable for fish (Figure 3.1). The hydropeaking events could be simulated up to 70 l.s^{-1}.

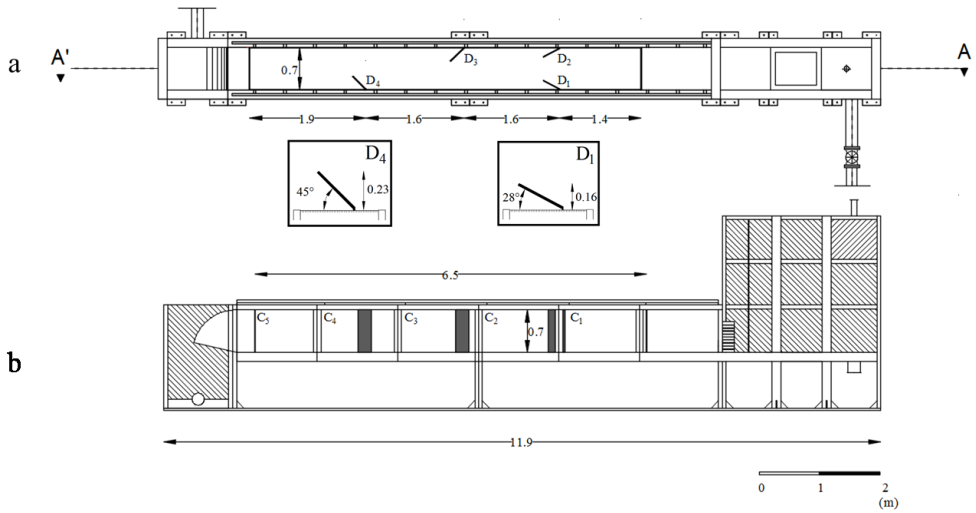

Figure 3.1 Top (a) and lateral (b) view of the indoor experimental flume with representation of the artificial refuges (deflectors: D1 to D4) and behaviour observation areas (C1 to C5). D1=D2 and D3=D4 in terms of opening angle relatively to the flume wall.

To mimic lateral refuges in a river channel, four PVC flashboards (0.30 m x 0.76 m x 0.015 m) herein termed as deflectors (D1-D4), were installed in the PVC false bottom of the flume (Figure 3.1). The deflectors were installed in a configuration characteristic of a meandering river reach, creating a more heterogeneous flow environment. The upstream deflectors were installed side-by-side, with a 28^{o} opening angle between the flume wall and the deflector, thus occupying 1/4 of the flume width (Figure 3.1, D1). This configuration allowed increasing water velocities in the area affected by the flashboards, creating a harsher hydraulic environment for fish. The other two deflectors located downstream on opposite walls, with a 45^{o} opening angle, occupied 1/3 of the flume width (Figure 3.1, D4). The remaining width allowed the fish to swim freely and to fully exhibit their swimming behaviour.

Hydropeaking events

The hydropeaking events were set according to usual operation procedures of a Portuguese hydropower plant (e.g. Boavida et al., 2015) in order to test whether the volume of water and the rapid changes over time would induce fish movement behaviour and promote significant changes in the blood physiology. The experimental design consisted of different hydropeaking events that combined flow magnitude and duration. The peak event flow ratio (magnitude) is defined as the maximum flow divided by the minimum flow (Sauterleute and Charmasson, 2014).). Given three tested discharges, i.e. two peak-flows (20 $l.s^{-1}$ and 60 $l.s^{-1}$) and a base-flow (7 $l.s^{-1}$), the flow ratios were 2.9 and 8.6, which are considered moderate to high (Harby and Noack, 2013; Sauterleute and Charmasson, 2014).

Duration was tested considering the sum of sequences of flow stimulus (base-flow followed by base-flow or base-flow followed by peak-flow) lasting 20 minutes each, resulting in three different total durations. The combination of flow magnitudes and durations resulted in four hydropeaking events with three total durations as follows: (i) single up-ramping (SgUR; treatments: Q_{20}, Q_{60}, Q_{base1}; 20 minutes); (ii) single up- and down-ramping (SgUDR; treatments: $Q_{20\text{-}base}$, $Q_{60\text{-}base}$, Q_{base2}; 20+20 minutes); (iii) step up-ramping (StUR; treatments: $Q_{20\text{-}60}$, Q_{base2}; 20+20 minutes); (iv) and repeated up- and down-ramping (RpUDR; treatment: $RQ_{60\text{-}base}$; 20+20+20+20 minutes) (Table 3.1,Figure 3.2). Q_{base1} and Q_{base2} were tested in the SgUR, and in SgUDR and StUR events, respectively to compare the physiology and movement behaviour changes of *L. bocagei* when subjected to hydropeaking events with a reference condition where hydropeaking was absent. The RpUDR event intended to test if doubling and quadrupling the total event duration for the 60 $l.s^{-1}$ flow conditions would affect the movement behaviour and blood physiology of *L. bocagei*.

Table 3.1 Description of the tested hydropeaking events. The treatments changed according to the tested discharges: base-flow (Q_{base}= 7 l.s^{-1}) and peak-flow (Q_{20} =20 l.s^{-1} and Q_{60} = 60 l.s^{-1}), and according to the sequences of 20 minutes discharge stimulus and total event duration. Before each hydropeaking event there was an acclimation period where *L. bocagei* were subjected to a 7 l.s^{-1} discharge for 30 minutes

Hydropeaking event	Treatment	Discharge sequences (l.s^{-1})	Total event duration (min) *
SgUR	Q_{20}	20	20
	Q_{60}	60	
	Q_{base1}	7	
SgUDR	$Q_{20\text{-}base}$	20-7	40
	$Q_{60\text{-}base}$	60-7	
	Q_{base2}	7-7	
StUR	$Q_{20\text{-}Q60}$	20-60	40
	Q_{base2}	7-7	
RpUDR	$RQ_{60\text{-}base}$	60-7-60-7	80

*excluding the 30 minutes acclimation.

Before starting a hydropeaking event, the flume discharge was set at Q_{base} for 30 minutes (acclimation period), maintaining the upstream gate open at a 10º angle and the downstream gate at 72º. To simulate a hydropeaking event, the upstream gate was closed while filling up the flume reservoir to its maximum capacity, and at the same time the discharge was manually controlled until attaining the tested peak-flow. Afterwards, the upstream gate was suddenly opened to a maximum of 10º while releasing the tested peak-flow. After each flow stimulus (20 minutes), the discharge was reduced to 7 l.s^{-1} or increased to 20 or 60 l.s^{-1} according to the tested event. The mean time (± SD) for up- and down-ramping was 28.12 ± 1.86', corresponding to an up-ramping rate of 0.55 cm.s^{-1}.

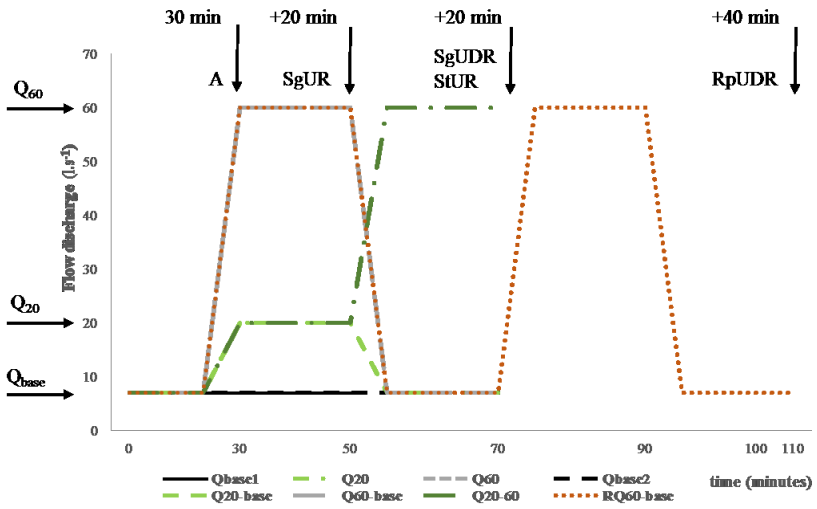

Figure 3.2 Hydropeaking events tested (SgUR, single up-ramping; SgUDR, single up- and down-ramping; StUR, step up-ramping; RpUDR, repeated up- and down-ramping) with indication of the duration (minutes) for each hydropeaking event (vertical black arrows; (30+20) for SgUR, (30+20+20) for SgUDR and StUR and (30+20+20+20+20) for RpUDR). The horizontal black arrows indicate the discharges tested (Q_{base}= 7 l.s^{-1}, Q_{20} = 20 l.s^{-1} and Q_{60} = 60 l.s^{-1}). The first 30 minutes (A) correspond to the acclimation period of *L. bocagei* in the flume. The mean time (± SD) for up- and down-ramping was 28.12 ± 1.86', corresponding to an up-ramping rate of 0.55 cm.s^{-1}.

Each treatment comprised a group of five *L. bocagei* and was replicated three times. Each fish was tested only once. The selected number of replicates and group size is currently accepted for this type of research (Auer et al., 2017; Branco et al., 2013). Bigger schools of *L. bocagei* likely occur in nature, particularly during the reproductive season, however, a school of five fish was selected to optimize the observation of fish movement behaviour in the flume, to reproduce a representative schooling behaviour (Weihs, 1973), and to reduce the number of fish to be collected from the wild.

Hydraulics

A detailed flow characterization was conducted using an Acoustic Doppler Velocimeter (ADV), the Nortek-AS Vectrino 10 MHz, with Vectrino Plus firmware. Three orthogonal velocity components were measured with a four-beam down-looking probe mounted on a fixed stem. This equipment measured the three components of flow velocity (*x, y, z*). The velocity components were measured at one horizontal plane set at a 5 cm water depth for 7, 20 and 60 $l.s^{-1}$. The reference grid consisted of 128 points with a maximum spacing interval of 10 cm at the deflector area and 15-50 cm at the remaining flume area. This mesh grid allowed a more refined characterization of the deflector area, where the most relevant fish movement behaviour and hydraulic characteristics were expected. The velocity measurements were performed at each point of the defined grid at a 100 Hz sampling rate and for a sampling period of 180 s, which is considered to be adequate for accurate velocity measurements (Buffin-Bélanger and Roy, 2005; Silva et al., 2011). The velocity magnitude was defined as $u_{mag} = \sqrt{\overline{u}^2 + \overline{v}^2 + \overline{w}^2}$, where u corresponds to the longitudinal (x-axis), v the transverse (y-axis), and w the vertical (z-axis) velocity directions. The velocity measurements were used to calibrate numerical models set up with the FLOW-3D® software (Flow Science Inc., 2012) (Figure 3.3). The numerical simulations allowed us to obtain detailed velocity characterizations for the base-flow and for both peak discharges (i.e. 20 and 60 ls^{-1}) (Figure 3.3). The FLOW-3D® numerical models were used as an additional tool to better understand the *L. bocagei* deflector approaches and flume movement behaviour according to the tested configurations.

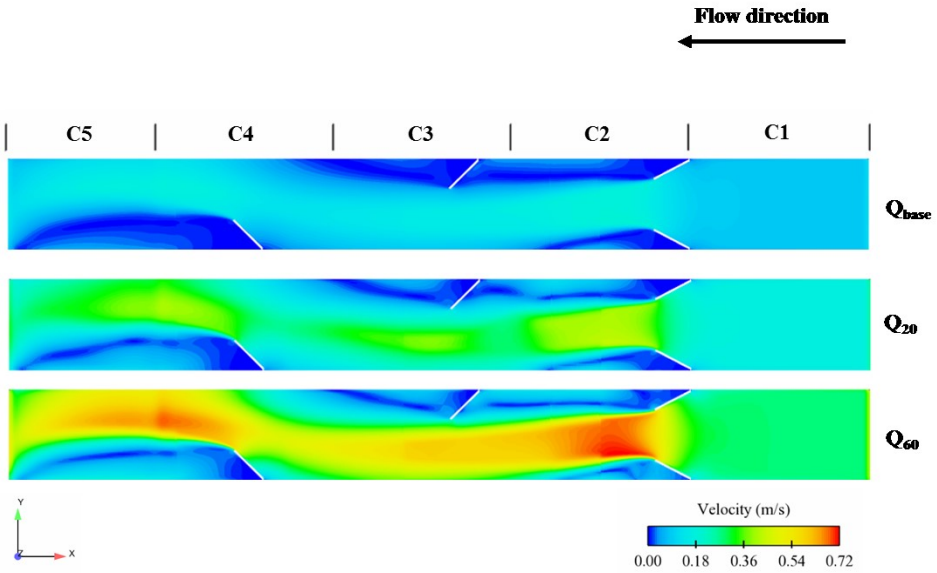

Figure 3.3 Velocity magnitudes for the tested discharges: base-flow (7 $l.s^{-1}$) and peak-flows (20 and 60 $l.s^{-1}$). C1 to C5 represent the five observation areas (results obtained with FLOW-3D®).

Fish responses

Physiological responses

Blood glucose and lactate concentrations were measured to assess physiological responses to stress. Glucose levels have been widely used as secondary physiological indicator of stress in flow variability research (Costa et al., 2017). Changes in this physiological indicator generally occur due to endocrine processes associated directly with primary responses to stress (Pankhurst, 2011). Blood lactate is tightly linked with muscle activity, and as a metabolic by-product of anaerobic exercise, it will likely increase when aerobic swimming is no longer sufficient to maintain sustained swimming activity (Wendelaar Bonga, 1997). To quantify these blood parameters, after a treatment each fish was dip-netted from the flume and transferred to a container with permanently oxygenated water and immediately placed in a v-shaped plastic trough in a supine position. Blood samples (0.1-0.5 ml) were collected via caudal puncture using 23 G or 25 G pre-heparinized needles within 3 minutes of capture. Laboratory studies have demonstrated that capture-related stress will not significantly influence cortisol concentrations if the sample is taken quickly (Sumpter, 1997). The levels of blood lactate and glucose were immediately measured using the portable meters Lactate Plus (Nova Biomedical UK) and Accu-check Aviva (Roche) respectively. Lactate and glucose portable meters have been confirmed to provide valid results (Beecham et al., 2006; Stoot et al., 2014; Tanner et al., 2010).

Movement behaviour

The behaviour metrics were divided into two categories: deflector approaches and movement in the flume. The behaviour frequency was measured as the number of occurrences of each behaviour pattern in periods of 20 minutes, consisting of one period for the SgUR event, two periods for the SgUDR and StUR events and four periods for the RpUDR event, according to the changes in flow stimulus. During each period, the deflector approaches were counted for each deflector (D1-D4), classified in downstream and upstream approaches, and registered as individual (I) or group (G) (i.e. 2 to 5 fish) approaches from downstream (Idown or Gdown) or upstream (Iup or Gup), respectively. Upstream and downstream approaches were directly associated with negative and positive rheotaxis, respectively. The flume movement metrics were selected for their potential to increase when fish were subjected to the selected hydropeaking events. Thus, the selected metrics were: fish sprints, as a sustained swimming activity, lasting a few seconds, characterized by several tail beats; fish drifts defined as voluntary or involuntary downstream fish displacements associated to drag; and jumps as sudden voluntary whole-body leaps outside the water. Sprints and drifts were registered for individuals (Isprint and Idrift) and groups (Gsprint and Gdrift). The behaviour occurrences were observed in five different areas (C1 to C5, Figure 1) and their frequency was registered for each individual (I) and group (G) (i.e. 2 to 5 fish) in each area, except for jumps. One behaviour occurrence was only assigned to a specific area (C1 to C5), and only if it started there. The fish movement behaviour observations were visually assessed by two observers who registered the occurrences on an ethogram sheet. One observation area was addressed to each observer: observer one monitored C1 to C3, and observer two C4 to C5. Each observer recorded all behaviour metrics. To avoid fatigue, after each experiment the observers rested during the acclimation period (30 minutes). The total observation period per day never exceeded 180 minutes. To avoid differences in behaviour observation results the two observers monitored the same area during pilot tests, and the movement behaviour results were considered satisfactory when there was an agreement for the movement behaviour results obtained by the observers.

Data analysis

A preliminary data exploration was conducted to assess outliers in the levels of physiological indicators of *L. bocagei* (n=5) for each replicate. If outliers were graphically detected, an interpretation of raw values was performed to verify if they corresponded to real outliers. If the levels of both blood lactate and glucose of *L. bocagei* were extreme in comparison with the rest of the individuals, the values were removed. This approach was used to avoid losing individual information that would not stand out if the raw data were transformed or mean values were used to follow the assumptions required to the

application of the parametric tests. Kruskal-Wallis tests were performed to test for statistical evidence that the levels of blood glucose and lactate between replicates differed. As there was not any statistical evidence that corroborated that hypothesis, the physiological responses of each *L. bocagei* were a true replicate. This preliminary analysis was followed by a Kruskal-Wallis test with a Nemenyi post-hoc test for pairwise contrasts, to check for differences in the blood physiology of *L. bocagei* between treatments of each experiment (SgUR, SgUDR and StUR) (Pohlert, 2015). In case there were ties between mean rank sums, the Chi-square approach was used (Pohlert, 2015). The Wilcoxon Rank sum test was used to compare the levels of physiological indicators of *L. bocagei* between the StUR event and the respective base-flow treatment and between the hydropeaking events with different durations (20 minutes *vs.* 40 minutes) where the 20 $l.s^{-1}$ discharge was applied.

A two-way distance-based multivariate analysis of variance based on Euclidean distance (Oksanen, 2015) was performed to test whether there was a treatment effect in the (1) frequency of deflector use and (2) flume movement behaviour metrics of *L. bocagei*. This method does not require the assumptions of parametric tests (Anderson, 2001), handles small samples (Walters and Coen, 2006) and both continuous and factor predictors (Oksanen, 2015). If an effect was detected, a detailed analysis per metric of deflector use and flume movement behaviour was performed using the Kruskal-Wallis test with a Nemenyi post-hoc test for pairwise contrasts (Pohlert, 2015). All statistical analysis were performed for α=0.05 with the RStudio Team software (RStudio inc, 2016).

3.1.4 Results

Hydraulics

The maximum flow velocity measured for 7 $l.s^{-1}$ was 0.18 $m.s^{-1}$ (Table 3.2) which is in accordance with the suitability curves for juveniles and adults (Martínez-Capel and García de Jalón, 1999) and the sustained swimming ability (Mateus et al., 2008). of *L. bocagei*. For peak-flows, velocities were 0.41 $m.s^{-1}$ and 0.71 $m.s^{-1}$ for the 20 $l.s^{-1}$ and the 60 $l.s^{-1}$ discharges respectively (Table 3.2). These hydraulic conditions were within the range for estimated critical swimming speeds for *L. bocagei* (Mateus et al., 2008) for young adults and also based on knowledge of velocity refuges in fishway passages (Silva et al., 2011).

As expected, the FLOW-3D® simulations showed that the lowest and the highest velocity ranges occurred in the 7 $l.s^{-1}$ and the 60 $l.s^{-1}$ discharges, respectively. The immediate area downstream of the

deflectors was characterized by the lowest flow velocities for the three simulated discharges (i.e. 7, 20 and 60 l.s^{-1}), and this was particularly evident for the 7 l.s^{-1} discharge (Figure 3.3).

Table 3.2 ADV results for longitudinal velocity and mean water depth for the tested discharges of 7, 20 and 60 l.s^{-1}, at 5 cm depth. The upstream and downstream velocities were collected in the downstream area of C1 and the upstream area of C5, respectively, where the ADV was fixed. The velocity magnitude is defined as $u = \sqrt{\overline{u}^2 + \overline{v}^2 + \overline{w}^2}$, where u corresponds to the longitudinal (x-axis), v the transverse (y-axis) and w the vertical (z-axis) velocity directions.

Discharge (l.s^{-1})	Velocity magnitude (m.s^{-1})			Water depth (cm)	
	Mean ± SD (m.s^{-1})	Maximum (m.s^{-1})	Minimum (m.s^{-1})		
				Upstream	Downstream
7	0.10±0.06	0.18	0.004	11.8	11.8
20	0.21±0.13	0.41	0.008	16.0	15.6
60	0.34±0.22	0.71	0.009	23.5	22.1

The harshest hydraulic conditions occurred when the highest peak-flow was tested and were prominent in the immediate downstream area between D1 and D2 in C2, and on the right area downstream D4 in C4 and C5 (Figure 3.3).

Physiological responses

There were significant differences in the blood glucose levels of *L. bocagei* in at least one treatment of the SgUR event (χ^2 (2) = 7.821, P = 0.020). Pairwise comparisons evidenced that this indicator differed significantly between Q_{60} and Q_{base1} (P = 0.021). The highest mean levels were observed in Q_{60} (61.1 ± 14.0 mg.dl^{-1}) and the lowest in the base-flow treatment (Q_{base1}: 45.6 ± 9.1 mg.dl^{-1}) (Figure 3.4). Contrarily, the mean blood lactate levels were lower in both the peak-flow treatments (Q_{20}: 3.05 ± 1.6 mM; Q_{60}: 3.06 ± 2.0 mM) than in Q_{base1} (4.09 ± 2.2 mM). However, there were no significant differences for this physiological indicator between treatments (χ^2 (2) = 2.089, P = 0.352). After being subjected to Q_{base2}, the mean blood glucose levels in *L. bocagei* were 55.1 ± 10.8 mg.dl^{-1}. The mean levels of this indicator were higher in *L. bocagei* subjected to the same duration peak-flow treatments, ranging from 58.5 ± 16.1 mg.dl^{-1} in $Q_{60\text{-}base}$, to 64.4 ± 11.4 mg.dl^{-1} in $Q_{20\text{-}60}$, and 65.1 ± 15.4 mg.dl^{-1} in $Q_{20\text{-}base}$ (Figure 3.4). For the StUR event, the blood glucose levels were significantly higher in $Q_{20\text{-}60}$ than in Q_{base2} (W = 162, P = 0.039). For the SgUDR event, blood glucose did not differ significantly between peak-flows and Q_{base2} (χ^2 (2) = 4.557, P= 0.102). Blood lactate levels ranged from 3.15 ± 2.1 mM in $Q_{60\text{-}base}$, and 3.42 ± 2.2 mM in $Q_{20\text{-}60}$, to 3.95 ± 2.0 mM in $Q_{20\text{-}base}$. The highest mean level of blood lactate (3.71 ± 2.1 mM) was registered for the same duration base-flow treatment (Q_{base2}). These differences were neither statistically significant between the StUR (W = 96.5, P = 0.519) nor the SgUDR (χ^2 (2). = 1.567, P= 0.457) events.

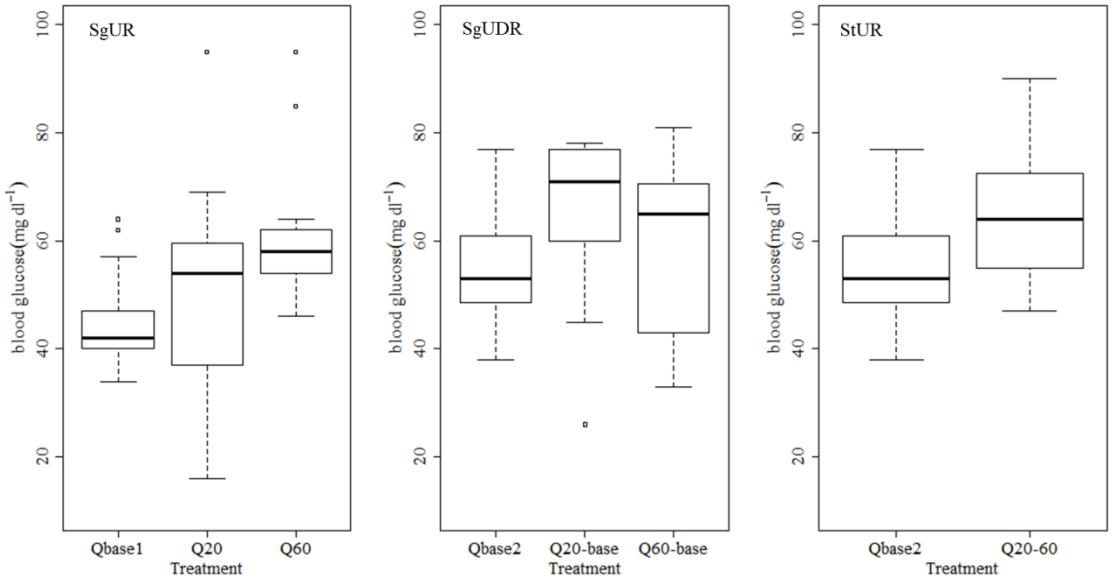

Figure 3.4 Boxplot indicating the variation of blood glucose levels (mg.dl^{-1}) between the treatments of the single up-ramping (SgUR), single up- and down-ramping (SgUDR) and step up-ramping (StUR) events.

For the 20 l.s^{-1} peak-flow, the mean blood glucose levels were 51.0 ± 19.2 mg.dl^{-1} after the 20 minutes treatment (Q_{20}, SgUR) and 65.1 ± 15.4 mg.dl^{-1} after the 40 minutes treatment ($Q_{20\text{-base}}$, SgUDR) (Figure 3.5 a and b), and this difference was statistically significant (W=46.5, P=0.009). Blood lactate levels did not differ significantly between these two treatments (W=83, P=0.342).

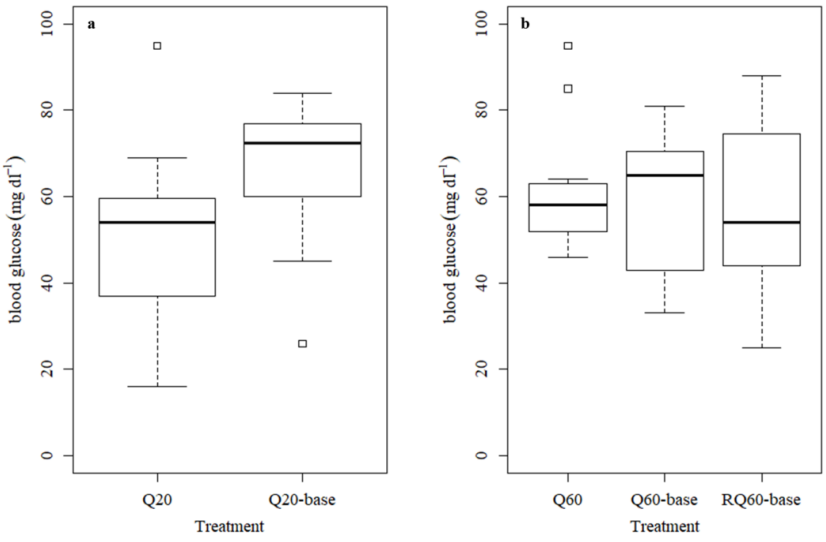

Figure 3.5 Boxplot indicating the variation of blood glucose levels (mg.dl^{-1}) for the different hydropeaking event durations: 20 (Q_{20}) vs. 40 minutes ($Q_{20\text{-base}}$) for the 20 l.s^{-1} discharge (a), and 20 (Q_{60}) vs. 40 ($Q_{60\text{-base}}$) vs. 80 minutes ($RQ_{60\text{-base}}$) for the 60 l.s^{-1} discharge (b).

For the 60 $l.s^{-1}$ peak-flow, there were no significant differences in the blood glucose levels (χ^2 (2) = 0.175, P = 0.916) of *L. bocagei* between the hydropeaking events that lasted 20 (SgUR: 61.1 ± 14.0 $mg.dl^{-1}$), 40 (SgUDR: 58.5± 16.1 $mg.dl^{-1}$) or 80 minutes (RpUDR: 58.3 ± 18.3 $mg.dl^{-1}$). The same pattern was verified for blood lactate levels (χ^2 (2) = 2.879, P = 0.237), where mean values ranged from 3.06 ± 2.03 mM, 3.15 ± 2.08 mM to 1.97 ± 1.1 mM, for the SgUR, SgUDR and RpUDR events respectively.

Movement behaviour

In general, the frequency of downstream deflector approaches was higher than upstream deflector approaches (Figure 3.6 a, c and e). The occurrences of sprints and drifts were more frequent when *L. bocagei* were subjected to 60 $l.s^{-1}$ and 20 $l.s^{-1}$ discharge conditions than to base-flow discharge treatments, with increased incidence for individual behaviour (Figure 3.6 b, d and f). The results from the multivariate analysis showed that for the 20 minute duration event (SgUR), there was a treatment effect on the deflector use by *L. bocagei* (F=3.821, P=0.005). Looking in detail for each deflector approach metric, Idown differed according to the discharge stimulus (χ^2 (2) = 11.611, P=0.003). Idown approaches were more frequent in Q_{20} in comparison with Q_{60} (Figure 3.6 a; Table 3.3). Deflector use did not differ between treatments for Gdown and both Iup and Gup (Table 3.3). Overall, deflector use was lower in Q_{60} (Figure 3.6 a). There was also a treatment effect in the frequency of sprints and drifts of *L. bocagei* (F=3.519, P=0.002); Gsprint (χ2 (2) = 14.18, P < 0.001), Idrift (χ2 (2) = 13.435, P = 0.001) and Gdrift (χ^2 (2) = 10.063, P = 0.006) differed significantly between the three flow treatments and were predominantly higher in Q_{60} and Q_{20} in comparison with Q_{base1} (Figure 3.6 b; Table 3.3). There was no statistical evidence for fish jumping more in Q_{60} and Q_{20} in comparison with Q_{base1} (Table 3.3). For the 40 minutes duration hydropeaking events (SgUDR and StUR), there was a treatment effect on the frequency of deflector use (F=3.389, P=0.001). Both Idown and Gdown differed significantly between treatments (χ^2 (3) = 15.679, P =0.001, and χ^2 (3) = 12.139, P =0.007 respectively). Pairwise comparisons showed that Idown were more frequent in the SgUDR ($Q_{60\text{-}base}$ and $Q_{20\text{-}base}$) and in the StUR ($Q_{20\text{-}60}$) events in comparison with Q_{base2} (Figure 3.6 c and e respectively). Gdown were more frequent only in the StUR event in comparison with Q_{base2} (Figure 3.6 c and e; Table 3.4). There was a treatment effect on the frequency of sprints and drifts of *L. bocagei* in the 40 minute duration hydropeaking events (F=11.273, P = 0.001). Isprint (χ^2 (3) = 29.815, P < 0.001), Gsprint (χ^2 (3) = 33.336, P < 0.001), Idrift (χ^2 (3) = 25.652, P < 0.001) and Gdrift (χ^2 (3) = 11.714, P = 0.008) differed significantly in at least one treatment.

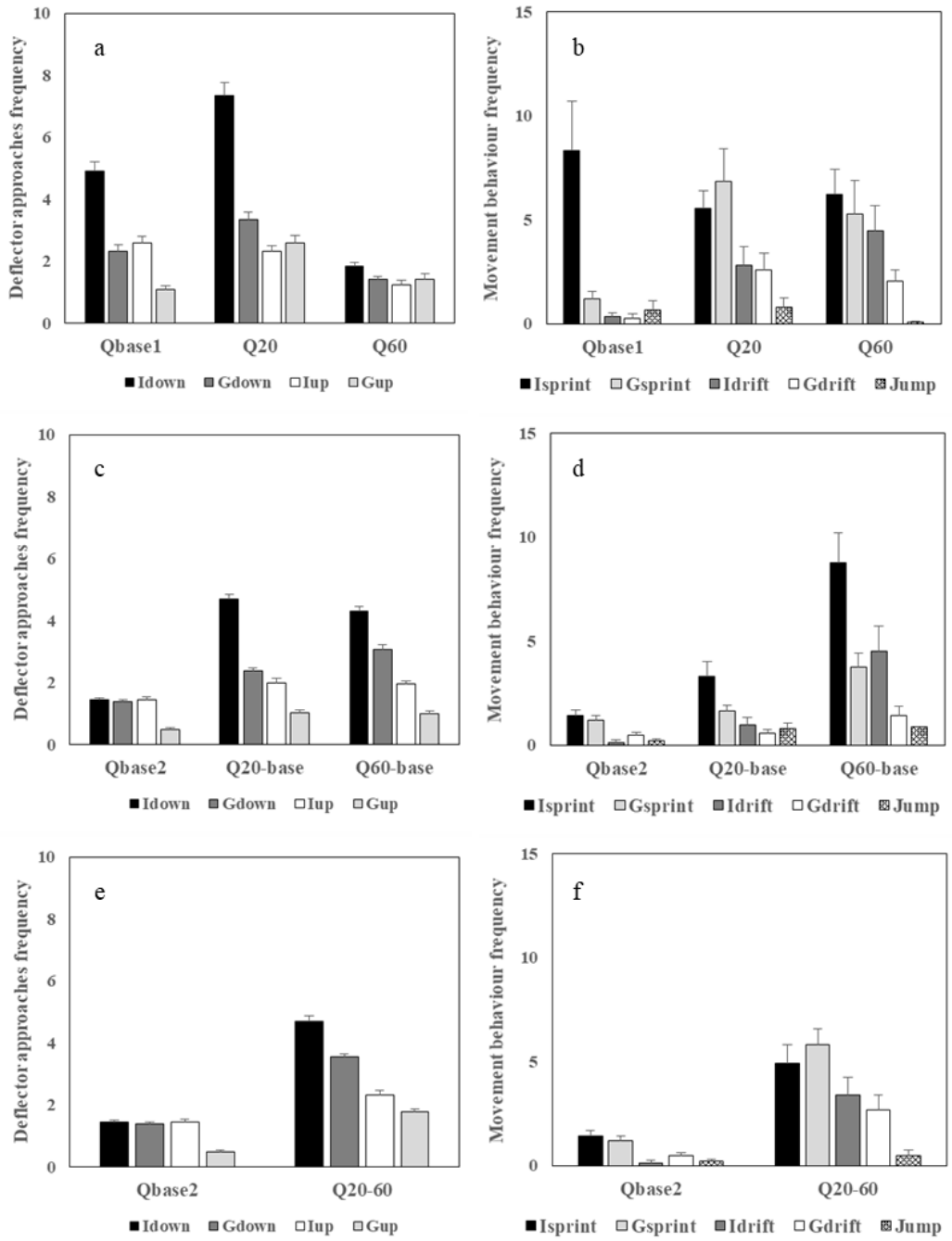

Figure 3.6 Mean (+ SE) frequency of occurrences for each behaviour metric referring to deflector approaches and movement behaviour respectively, for (a) and (b) single up-ramping (SgUR), (c) and (d) single up- and down-ramping (SgUDR), and (e) and (f) step up-ramping (StUR) hydropeaking events.

Pairwise comparisons revealed that Isprint increased in the SgUDR ($Q_{60\text{-}base}$) and StUR events in comparison with Q_{base2} (Figure 3.6 d and f respectively; Table 3.4), and also in $Q_{60\text{-}base}$ in comparison with $Q_{20\text{-}base}$ (Figure 3.6 d; Table 3.4). Gsprint were more frequent in the SgUDR event ($Q_{60\text{-}base}$) in comparison with Q_{base2}, and in the StUR event in comparison with both the SgUDR event ($Q_{20\text{-}base}$) and Q_{base2} (Figure

3.6 d and f respectively; Table 3.4). Idrift increased in the SgUDR ($Q_{60\text{-}base}$) and in the StUR events in comparison with Q_{base2} (Figure 3.6 d and f; Table 3.4). Gdrift were more frequent only in the StUR event in comparison with Q_{base2} (Figure 3.6 f; Table 3.4).

Table 3.3 Single up-ramping (SgUR) results for the pairwise comparisons between peak (Q_{20} = 20 l.s^{-1} and Q_{60} = 60 l.s^{-1}) and base (Q_{base1} = 7 l.s^{-1}) flow treatments, according to deflector approaches and flume movement behaviour, using the Kruskal-Wallis test with a Nemenyi post-hoc test for pairwise contrasts. Significant results (α= 0.05) are indicated in bold.

Pairwise comparisons		**Deflector approaches**				**Flume movement behaviour**				
		Downstream		Upstream		Sprints		Drifts		Jumps
		Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
Q_{20} vs. Q_{base1}	χ^2	1.175	0.754	0.00089	1.549	4,877*10^{-5}	**13.515**	**6.1899**	**7.2937**	0.0194
	P	0.556	0,69	0.42	0.46	1.00	**0.001**	**0.045**	**0.026**	0.99
Q_{60} vs. Q_{base1}	χ^2	5.088	0.437	1.732	0.003	1.951*10^{-2}	**6.475**	**12.781**	**7.7924**	1.782
	P	0.078	0.80	0.42	1.00	0.99	**0.039**	**0.002**	**0.026**	0.41
Q_{20} vs.Q_{60}	χ^2	**11.153**	2.341	1.654	1.424	1.760*10^{-2}	1.281	1.1818	0.00824	2.174
	P	**0.004**	0.31	0.44	0.49	0.99	0.527	0.554	0.996	0.34

In the SgUDR event for both peak-flows tested, flume activity decreased in the last 20 minutes of the hydropeaking event, where a 7 l.s^{-1} discharge was applied (Figure 3.7 b).

Table 3.4 Single up- and down-ramping (SgUDR) and step up-ramping (StUR) results for the pairwise comparisons between peak ($Q_{20\text{-}base}$, $Q_{60\text{-}base}$ and $Q_{20\text{-}60}$) and base (Q_{base2}) flow treatments for each behaviour according to deflector approaches and movement behaviour, using the Kruskal-Wallis test with a Nemenyi post-hoc test for pairwise contrasts. Significant results (α= 0.05) are indicated in bold.

Pairwise comparisons		**Deflector approaches**				**Flume movement behaviour**				
		Downstream		Upstream		Sprints		Drifts		Jumps
		Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
$Q_{20\text{-}base}$ vs. Q_{base2}	χ^2	**12.032**	3.095	0.217	0.442	2.685	0.981	2.698	0.148	0.664
	P	**0.007**	0.377	0.97	0.93	0.443	0.806	0.441	0.986	0.88
$Q_{60\text{-}base}$ vs. Q_{base2}	χ^2	**8.587**	3.847	1.852	0.458	**27.952**	**8.359**	**18.141**	1.095	0.004
	P	**0.035**	0.278	0.60	0.93	**<0.001**	**0.039**	**<0.001**	0.778	1.00
$Q_{20\text{-}60}$ vs. Q_{base2}	χ^2	**10.304**	**12.068**	0.545	7.052	**8.383**	**28.643**	**17.283**	**9.825**	0.032
	P	**0.016**	**0.007**	0.91	0.07	**0.039**	**<0.001**	**<0.001**	**0.020**	1.00
$Q_{20\text{-}base}$ vs. $Q_{20\text{-}60}$	χ^2	0.0670	2.940	0.074	3.961	1.580	**19.021**	6.324	7.562	0.991
	P	0.995	0.401	0.99	0.27	0.664	**<0.001**	0.097	0.056	0.80
$Q_{60\text{-}base}$ vs. $Q_{20\text{-}60}$	χ^2	0.078	2.287	0.387	3.915	5.720	6.055	0.010	4.361	0.014
	P	0.994	0.515	0.94	0.27	0.126	0.109	0.999	0.225	1.00
$Q_{20\text{-}base}$ vs. $Q_{60\text{-}base}$	χ^2	0.290	0.041	0.800	0.00013	**13.310**	3.612	6.847	0.438	0.771
	P	0.962	0.998	0.85	1.00	**0.004**	0.306	0.077	0.932	0.86

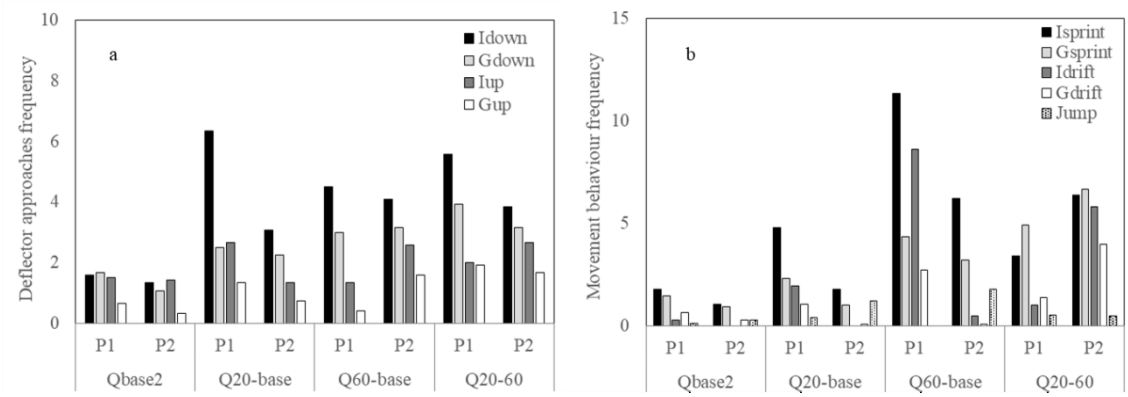

Figure 3.7 Mean frequency of occurrences for each behaviour metric referring to deflector approaches (a) and movement behaviour (b), for each 20 minutes duration flow stimulus (P1 vs. P2) of the 40 minutes duration hydropeaking events: SgUDR ($Q_{20\text{-}base}$, $Q_{60\text{-}base}$) and StUR ($Q_{20\text{-}60}$) and respective base-flow treatment (Q_{base2}).

This difference was particularly evident in $Q_{60\text{-}base}$ for Isprint and Idrift (Figure 3.7 b). Conversely, in the second period of the StUR event (60 $l.s^{-1}$), the frequency of movement behaviour increased, particularly fish drifts (Figure 3.7 b). For the 20 $l.s^{-1}$ peak-flow treatments, there was no effect of total event duration on the deflector use by *L. bocagei* (F=2.466, P=0.065). On the other hand, the behaviour frequency of *L. bocagei* was affected by total event duration in the flume (F=1.097, P=0.001).Pairwise comparisons evidenced that these differences occurred in Isprint, Gsprint, Idrift and Gdrift (Table 3.5), and except for Isprint, they were higher in the shortest duration event.

Table 3.5 Comparisons between total hydropeaking event duration for 20 and 60 $l.s^{-1}$ respectively. The comparisons were performed to test for differences between treatments for each deflector approach and movement behaviour metric, using the Kruskal-Wallis test with a Nemenyi post-hoc test for pairwise contrasts for the 60 $l.s^{-1}$ treatments, and with Wilcoxon Rank sum test for the 20 $l.s^{-1}$ treatments. Significant results (α= 0.05) are indicated in bold

Peak discharge ($l.s^{-1}$)	**Comparisons between treatments**		**Deflector approaches**				**Flume movement behaviour**				
			Downstream		Upstream		Sprints		Drifts		Jumps
			Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
20	Q_{20} vs. $Q_{20\text{-}base}$	W	184.5	164	171.5	194	**313.3**	**384.5**	**310**	**311.5**	233
		P	0.175	0.507	0.342	0.069	**0.027**	**<0.001**	**<0.001**	**<0.001**	0.842
60	Q_{60} vs.$Q_{60\text{-}base}$	χ^2	4.679	2.154	1.393	0.320	0.859	0.333	0.765	2.362	0.406
		P	0.096	0.341	0.50	0.85	0.65	0.846	0.68	0.31	0.82
	Q_{60} vs. $RQ_{60\text{-}base}$	χ^2	**8.499**	**7.278**	2.477	0.448	0.411	1.822	2.125	0.099	2.555
		P	**0.014**	**0.026**	0.29	0.80	0.81	0.402	0.35	0.95	0.28
	$Q_{60\text{-}base}$ vs. $RQ_{60\text{-}base}$	χ^2	0.496	1.980	0.131	2.768	4.572	6.548	0.416	3.125	1.353
		P	0.780	0.371	0.94	0.25	0.10	0.038	0.81	0.21	0.51

When the flow stimulus was set at 60 $l.s^{-1}$, event duration affected the deflector use (F=2.789, P=0.007). Idown (χ^2 (2) = 8.502, P = 0.014) and Gdown (χ^2 (2) = 7.819, P = 0.020) differed significantly in at least one hydropeaking event duration (i.e. 20 (SgUR), 40 (SgUDR) or 80 (RpUDR) minutes) (Table 3.5). The

downstream approaches were higher in the RpUDR event in comparison with the SgUR event for both individuals and groups (Figure 3.8).

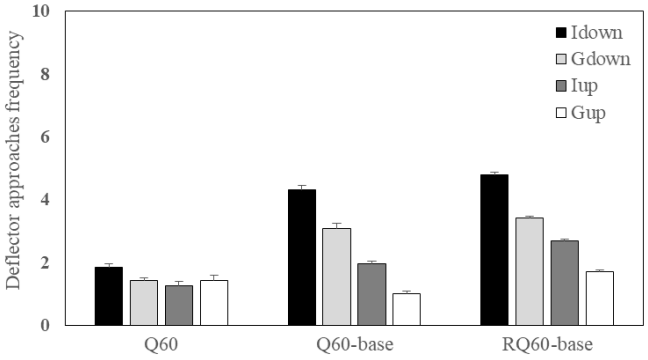

Figure 3.8 Mean (+ SE) frequency of occurrences for each behaviour metric referring to deflector approaches for the treatments with 60 $l.s^{-1}$ peak discharge: Q_{60} (SgUR, 20 minutes), $Q_{60\text{-}base}$ (SgUDR, 40 minutes) and $RQ_{60\text{-}base}$ (RpUDR, 80 minutes).

On the other hand, there was no statistical evidence that upstream deflector approaches (i.e. Iup and Gup) differed between these hydropeaking events (Table 3.5). Rank comparisons evidenced that Gsprint differed significantly between the three hydropeaking events (χ^2 (2) = 7.057, P = 0.029) (Table 3.5), which were higher in the RpUDR event in comparison with the SgUDR. Isprint and Idrift were always higher in the time periods where fish were affected by the 60 $l.s^{-1}$ discharge, in opposition to the 7 $l.s^{-1}$ discharge (Figure 3.9), and although group behaviour also increased, it was not as evident as individual behaviour. In the RpUDR event in the last two stages there was a decrease in flume activity in comparison to the first two stages, although it was not statistically significant (Figure 3.9).

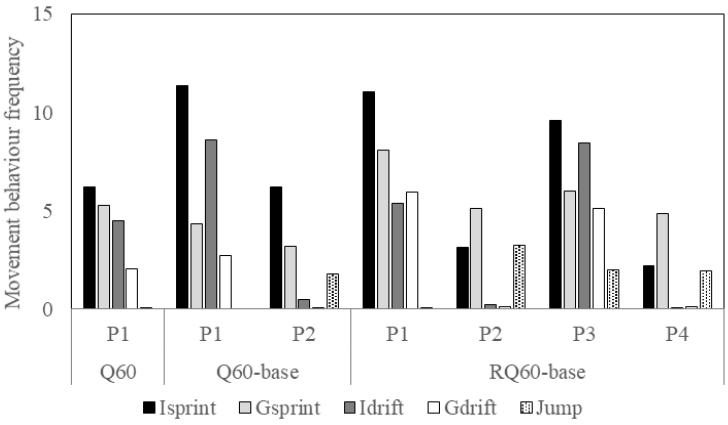

Figure 3.9 Mean frequency of occurrences of each movement behaviour, i.e. sprints (Isprint and Gsprint), drifts (Idrift and Gdrift) and jumps (Jump), for each 20 minutes timespan flow stimulus (P1, P2, P3 and P4), and for the 60 $l.s^{-1}$ discharge treatments: Q_{60} (single up-ramping), $Q_{60\text{-}base}$ (single up- and down-ramping), and $RQ_{60\text{-}base}$ (repeated up- and down-ramping).

3.1.5 Discussion

In the present study we assessed the physiological and behavioural consequences of simulated hydropeaking events for *L. bocagei* in the presence of velocity refuges. The increased levels of blood glucose observed in fish exposed to the highest peak-flow, i.e. Q_{60} (SgUR), and in the event with the longest duration peak-flow stimuli, i.e. $Q_{20\text{-}60}$ (StUR), enable us to reject the null hypothesis that flow magnitude and hydropeaking event duration do not affect the blood physiology of *L. bocagei*. However, simulated hydropeaking events failed to elicit significant changes in blood lactate. In general, the velocity refuges were used more frequently during the peak-flows in comparison with base-flows; downstream approaches were the most frequent, and individual approaches stood out particularly in the 20 $l.s^{-1}$ flow stimulus. The available flume area was used differently between peak-flow and base-flow treatments. Sprints and drifts were more frequent in the peak-flow treatments, and in particular during the peak-flow stimulus. These results enable us to reject the null hypothesis that velocity refuges and flume behaviour are used equally by *L. bocagei* under peak-flows and base-flows.

The more unstable hydraulic conditions and higher flow velocities in the 60 $l.s^{-1}$ conditions, obtained with the FLOW-3D® numerical model (Figure 3.3), and the unpredictability of the StUR event with the potential cumulative effect of the two tested peak-flows, i.e. 20 $l.s^{-1}$ followed by 60 $l.s^{-1}$, were not favourable for *L. bocagei* to successfully find the velocity refuge. These factors may explain the increased levels of blood glucose and the lowest overall deflector approaches in Q_{60} (SgUR) (Figure 3.6 a). The similar levels of blood glucose between Q_{base2}, $Q_{20\text{-}base}$ and $Q_{60\text{-}base}$, (Figure 3.4 SgUDR) together with the increased individual downstream deflector approaches when fish were subjected to 20 $l.s^{-1}$ in both the SgUR (Figure 3.6 a) and SgUDR (Figure 3.7) events, indicate that hydropeaking events with short duration peak-flow stimulus and moderate flow ratios create advantageous conditions for this species to find a low flow refuge, reducing the chances of an acute physiological response.

The increased levels of blood glucose in $Q_{20\text{-}base}$ in comparison with Q_{20} (Figure 3.5 a) suggest that event duration also affects the blood physiology of *L. bocagei*. After 40 minutes timespan there seemed to be a peak in the blood glucose response. However, this trend was not verified in the levels of blood glucose between *L. bocagei* subjected to Q_{60}, $Q_{60\text{-}base}$ and $RQ_{60\text{-}base}$ (Figure 3.5 b). The similar high levels of blood glucose between these treatments suggest that the glucose peak lasted for at least 80 minutes. The less severe 20 $l.s^{-1}$ flow conditions possibly resulted in a slower increase in the blood glucose response, in opposition to the early peak shown after 20 minutes under the harsher 60 $l.s^{-1}$ discharge. Different stressor intensities and combinations seem to result in different physiological responses over time. For example, earlier and more prolonged glucose and lactate responses were observed in European chubs

affected by electrofishing in comparison to handling (Bracewell et al., 2004). Higher ranges of glucose levels than those reported here, were found in *L. bocagei* that were exposed to a combination of chemical pollution and confinement for 2 h in the Tajo river (Carballo et al., 2005). In experiments where the effects of handling, acclimation time and hydropeaking (flow reduction) were assessed in juvenile brown trout, peak levels of blood glucose were observed immediately after electrofishing (i.e., capture), levelled off after 72 hours of acclimation, and increased again after dewatering (Arnekleiv et al., 2004). The combination of other stressors (nutritional status, temperature and fish density) could also have contributed to the sustained blood glucose response 24 hours after flow reduction (Arnekleiv et al., 2004). However, responses to flow are not universal. Juvenile brown trout subjected to flow reduction showed no effect on the blood glucose response (Flodmark et al., 2002). These studies highlight that the stressor severity, the timespan from which it was applied to sampling, and fish condition influence the responses to stress.

The relative stability of blood lactate levels suggests that the flow stimulus was not vigorous enough to promote anaerobic energy production through lactate accumulation (Gleeson, 1996), or that the timespan from flow stimulus to blood collection was insufficient to observe an elevation in blood lactate (Gleeson, 1996). The stability between the glucose response in the SgUDR treatments, its persistence between the three 60 $l.s^{-1}$ duration events, and the low lactate response could denote a sub-organismal and behavioural adjustment to conserve energy through compensation. This adjustment was favoured by the short duration 60 $l.s^{-1}$ flow stimulus and the low velocity areas inside and in the proximity of the downstream area of the deflectors (Figure 3.3).

The repeatability of the $RQ_{60\text{-}base}$ resulted in similar glucose levels in comparison with the same peak-flow treatments (i.e., Q_{60} and $Q_{60\text{-}base}$) (Figure 3.5 b). Longer simulated hydropeaking events could possibly result in habituation, with no physiological or behavioural changes over time. Similar results were obtained by Krimmer et al. (2011) and Flodmark et al. (2002), where the presence of refuges in hydropeaking rivers and in simulated down-ramping indoor experiments, might have alleviated the stressor severity resulting in a less pronounced stress response in brook trout and in brown trout respectively. These studies were conducted in hydropeaking rivers (Krimmer et al., 2011) and in stream channels (Flodmark et al., 2002) where it was difficult to directly observe fish behaviour, reinforcing the importance of performing indoor flume experiments to validate these explanations. If there was a sustained simultaneous lactate and glucose response, caused by the extreme flow conditions, exhaustion would possibly occur and fish would no longer be able to respond to the flow stimulus (Flodmark et al., 2002) and to regain homeostasis. In nature these responses would likely become maladaptive with deleterious effects on the fishes overall condition, growth, reproduction, behaviour

and disease resistance, and would likely have impacts at the population level (Barton, 2002; Pankhurst, 2011).

The effects of flow magnitude and hydropeaking event duration were more evident in the behavioural aspects of the study (overall deflector approaches and flume activity) than for the physiological metrics we used. Those effects were particularly evident in $Q_{20\text{-}60}$ (StUR) and $Q_{60\text{-}base}$ (SgUDR) (Figure 3.6). The deflector approaches were more frequent in the peak-flow treatments, for both peak-flows tested (Figure 3.6 a, c and e). Similarly, in a simulated pulsed-flow study conducted in an experimental flume, under increasing flow velocities three juvenile stream fishes (cyprinid, salmonid and catastomid) swam faster, and also hid in the available rocky substrate, presumably to conserve energy (Chun et al., 2011). Under maximum velocities up to 0.46 $m.s^{-1}$, those juveniles were able to maintain their swimming speed and hold position (Chun et al., 2011), whereas in the present study those activities were possible under velocities up to 0.72 $m.s^{-1}$. Both the presence of deflectors and the shorter duration peak-flow stimulus in the present study may have ameliorated the potential negative effects of higher velocities. Exceptionally, there was a low frequency of deflector approaches in Q_{60} (SgUR), explained by the existing critical hydraulic conditions (Figure 3.3) perceived by *L. bocagei*. Attempting to cope with the flow disturbance and high water velocities (Figure 3.3), it became challenging for fish to use the deflectors as low flow areas to conserve energy and to recover. These results were in agreement with the increased glucose levels shown in this hydropeaking event, indicating that *L. bocagei* are possibly experiencing stressful flow conditions, which resulted in an early glucose peak after 20 minutes of exposure. In contrast to the 20 $l.s^{-1}$ and the 7 $l.s^{-1}$ flow conditions, these results indicate that the most favourable hydraulic conditions were exceeded when *L. bocagei* were subjected to a discharge of 60$l.s^{-1}$, compromising their overall swimming performance. In a study where velocity refuge, water temperature and season were combined in simulated fluctuating flow conditions, brown trout moved to velocity refuges due to displacement, to avoid the first high flow winter periods (Vehanen et al., 2000) , however, the water velocities that the fish experienced were lower than those registered in this study.

In the 40-minute events, *L. bocagei* sought the deflectors more actively under peak-flows, individually and from downstream in comparison with Q_{base2} (Figure 3.6 c and e). Similar results have been reported for juvenile brown trout, which preferred to approach lateral refuge configurations from downstream under hydropeaking conditions (Ribi et al., 2014). Generally, fish tend to avoid demanding hydraulic conditions, choosing low-flow areas, i.e. flow refuging (e.g. Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012; Vehanen et al., 2000). However, when discriminating each flow stimulus period, the difference in the frequency of deflector approaches was less evident for $Q_{60\text{-}base}$ (SgUDR) (Figure 3.7 a) and there was a lower deflector use in the last 20 minutes of $Q_{20\text{-}60}$ (StUR) (Figure 3.7 a). These results

could be explained by the critical hydraulic conditions created in the vicinity of the deflector area in the 60 $l.s^{-1}$ flow stimulus (Figure 3.3), limiting this cyprinid's ability to seek out velocity refuge inside and downstream of the deflector.

In the natural environment, *L. bocagei* often occurs in schools possibly to benefit from the external hydrodynamic stimuli and to reduce the energetic costs associated to swimming activity (Bleckmann and Zelick, 2009; Liao, 2007). Under the highly modified hydropeaking conditions that were tested, a group behaviour disruption and an increase in the individual activity of *L. bocagei* were expected. The difference between group and individual approaches during the 20 $l.s^{-1}$ flow stimulus (Figure 3.7 a) could be explained by the lower energy required to hold station when subjected to this discharge (Scruton et al., 2008), in comparison with the more severe hydraulic conditions experienced during the 60 $l.s^{-1}$ flow stimulus. In addition, the higher flow velocities existing under the 20 $l.s^{-1}$ discharge, in comparison with the 7 $l.s^{-1}$ flow conditions, and the smaller size of young adults, possibly favoured the relative performance (Mateus et al., 2008) and rheophilic behaviour (Rodriguez-Ruiz et al., 1992) of *L. bocagei*. Although this species usually occurs in schools, the flow velocities and hydraulic conditions created (Figure 3.3) either promoted the disruption of group behaviour and facilitated the individual access to the deflectors as recovery areas, or created hydraulic conditions that favoured these young adults to freely use all available areas in the flume. Changes in social interactions due to fluctuating flows in artificial tanks have been reported for salmonids (Sloman et al., 2002, 2001) , resulting in lower growth rates of the less dominant fish (Sloman et al., 2002) , whereas in constant flow conditions the hierarchical structure remained stable. The alteration in social interactions, with consequent reduced growth rates in the less dominant fish (lower ability to access food), and the higher energy costs of holding station to negotiate with the flow disturbance, likely to occur following hydropeaking, may have deleterious consequences for subsequent life-cycle events, namely reproduction or migration. The reduced availability of low flow habitats in these highly instable flow environments will likely hinder the fishes' capacity to successfully persist under hydropeaking conditions.

The hypothesis that flow magnitude affected the movement behaviour of *L. bocagei* was supported by the increased frequency of sprints and drifts in peak-flows in comparison with the base-flow stimulus (Figure 3.6 b, d and f) and in the time periods where *L. bocagei* were subjected to the 60 $l.s^{-1}$ flow conditions (Figure 3.7 b and Figure 3.9). The increased individual sprints during the 20 minutes where *L. bocagei* were subjected to 60 $l.s^{-1}$ discharge (Figure 3.7 b) suggest that group behaviour could be disrupted. In nature, this species characteristically occurs in schools, benefiting from the surrounding hydraulic conditions to spend less energy (Liao, 2007; Sfakiotakis et al., 1999), thus the disruption of social behaviour caused by fluctuating, unpredictable flows could affect diel activity and crucial life-cycle

stages. This effect has been demonstrated in fluctuating flow environments in experimental flumes (Sloman et al., 2002, 2001).

The higher frequency of sprints suggests that *L. bocagei* were coping with the perceived flow velocities favouring positive rheotaxis. The increased frequency of drifts (either voluntary or involuntary) suggests that this could be a mechanism for *L. bocagei* to recover from the additional effort necessary for holding station, which is a function of the fish drag multiplied by the water velocity (Webb, 1988), due to the severe hydraulic conditions created, particularly in the 60 $l.s^{-1}$ discharge conditions. The more time *L. bocagei* spends coping with the severe flow conditions, for example sprinting or holding station, the less energy will be available for diel activities, namely foraging (Flodmark et al., 2004) or avoiding predators (Korman and Campana, 2009).

The presence of deflectors in a fluctuating flow environment with a moderate flow ratio (Q_{peak}/Q_{base}), i.e. 2.9, is expected to improve the swimming performance of this species at this life-stage. This was demonstrated by the lower levels of blood glucose in *L. bocagei* for the base-flow and the shorter duration peak-flow stimulus events, and by the favourable hydraulic conditions created in the 20 $l.s^{-1}$, which allowed *L. bocagei* to swim freely and easily reach velocity refuges. In opposition, the increased levels of blood glucose and the frequency of movement behaviour in the StUR event, indicate that hydropeaking events characterized by prolonged peak-flow sequences are not beneficial for *L. bocagei*, thus alternating short duration timespan peak-flows with base-flows is advisable. The increased frequency of deflector approaches in the 60 $l.s^{-1}$ treatments and the lower changes in the physiological levels in the $Q_{60\text{-}base}$ and $RQ_{60\text{-}base}$ events suggest that the presence of deflectors does provide refuge from high flows. Nevertheless, the simultaneous lower deflector use in these treatments in comparison with the lower peak-flow treatments, and the increase in the overall movement behaviour, suggest that this flow ratio and the critical hydraulic conditions created in the vicinity of the deflectors (Figure 3.3), seem to decrease the capacity of *L. bocagei* to find the flow refuges downstream of the deflectors.

This study demonstrated that the duration and the sequences of peak-flows together with flow ratio, created distinct flow patterns, resulting in varied behavioural responses of fish. Likewise, in rivers affected by hydropeaking, the short-term water level and velocity fluctuations alter fish behaviour in diverse ways. Hence, before defining the dimensions of the deflectors, it is necessary to identify the river hydromorphological changes caused by hydropeaking that will alter channel morphology (Schmutz et al., 2015), and the extent to which the added habitat heterogeneity (deflectors) might create unstable hydraulic conditions for fish (Auer et al., 2017). Hauer et al. (2017) used numerical simulations to demonstrate that in river channels influenced by hydropeaking, more heterogeneous habitats with

alternating gravel bars created a more unstable flow environment than when compared to reaches that only contained point bars. Therefore, the dimensions, spatial arrangement and number of the proposed deflectors should be assessed according to the rivers' hydromorphology, biological processes and societal demands (e.g. Woolsey et al., 2007). Although the flume was 0.7 m wide, it was possible to manipulate the dimensions of the deflectors, i.e. their opening angle and length, according to the size of young adults of *L. bocagei*. In a natural context, the deflectors would have to be adapted to the river width in order to provide habitat conditions similar to the simulated in this study. If they were simply scaled-up, this would result in oversized refuges. According to our findings and to fish passage studies with *L. bocagei* (Santos et al., 2014), the distance from the river bank to the edge of the deflector (determined by the opening angle of the deflector) should be at least in the same order of magnitude as the fish body length, thus not requiring overly wide angles in relation to the river bank. However, the fact that this species often occurs in schools should also be taken into consideration. In rivers affected by hydropeaking, the proposed opening angle would guide the flow, reducing the deposition of fine sediment in contrast with wider angled structures (Hauer et al., 2017), and clogging associated with accumulated driftwood (Ribi et al., 2014). Peak flows also create turbulent conditions, particularly in the vicinity and in the downstream edge of the deflectors, from which fish can benefit (Liao, 2007; Santos et al., 2014), likely increasing their attraction to the deflectors. To avoid fish stranding during the critical down-ramping phase, it should be guaranteed that the area behind the deflectors would not allow the formation of potential stranding zones, or assure a minimum water depth of 0.5 m behind them (Almeida et al., 2017; Ribi et al., 2014).

3.1.6 Conclusions

This study provided new insight on the effects of hydropeaking events on the physiology and movement behaviour of *L. bocagei*. The physiological changes and the increased movement behaviour of *L. bocagei* in the indoor experimental flume demonstrated that this species was affected by flow magnitude and hydropeaking event duration. The presence of deflectors, mimicking natural lateral refuges, increased the flume's morphological heterogeneity and provided alternative velocity options, alleviating the severity of the peak-flow conditions. The less harsh hydropeaking events characterized by lower peak-flows, i.e. 20 $l.s^{-1}$, were favourable for maintaining the homeostasis of this species. The unstable hydraulic conditions created during the 60 $l.s^{-1}$ flow stimulus appear to have reduced the fishes' ability seek out the deflectors as velocity refuges. These hydropeaking conditions will affect the downstream populations of *L. bocagei* as the energy available for diel activities (foraging, avoiding predators) and life-cycle stages (growth, reproduction), will be reduced. In addition, as this species naturally occurs in schools, the consequent disruption of social behaviour will also affect the energy

budget, as the beneficial hydraulic consequences of group behaviour will decrease. The evident increase in the frequency of individual downstream deflector approaches when *L. bocagei* were subjected to the 20 $l.s^{-1}$ discharge indicates that the presence of deflectors favours energy conservation due to the increased availability of velocity refuges, and that the hydraulic conditions created during the lower peak-flows are favourable for this cyprinid to find low velocity areas. Future experimental research is recommended to understand whether an increase in the severity of hydropeaking events results in habituation or exhaustion. The increasing trend for energy production using hydropower, the continuous alteration of freshwater ecosystems downstream hydropower plants and the need to develop new strategies to mitigate the impacts of these structures are strong arguments to conduct these types of studies. Finally, the use of an integrated approach strengthened our findings, contributed to an increase in knowledge about the impacts of hydropower in fish, and served as a model for future mitigation studies for fish.

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3.1.9 References

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3.2 The potential of lateral refuges for Iberian barbel under simulated hydropeaking conditions

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3.2.1 Highlights

- Hydropeaking events and deflector use were investigated for the Iberian barbel
- Higher velocities (≈ 0.6 $m.s^{-1}$) and flow ratios (5.7 and 8.6) promoted deflector use
- Individual deflector use and swimming activity predominated in the single-step event
- In the presence of deflectors peak repetition triggered a glucose response
- Movement behaviour generally decreased during the second peak with deflectors

3.2.2 Abstract

Fish responses to hydropeaking may range from sub-organismal adjustments to key life-event changes. It is currently difficult to quantify these responses and to assess the potential of artificial refuges accordingly. To address this problem, two experimental trials were conducted in an indoor flume. In the first, two hydropeaking events were tested in the presence of deflectors as flow refuges. In the second, the presence and absence of deflectors was tested for the same hydropeaking event. A multidisciplinary approach combined physiological and movement behaviour responses of Iberian barbel (*Luciobarbus bocagei*) with hydrodynamic models. These were used as a complementary tool to explain the fish responses. In the first experiment, no evident physiological adjustments occurred. However, individual refuge use and swimming activity were higher in the single-step event where velocities above 0.6 $m.s^{-1}$ were observed. In the second experiment, the levels of glucose were the highest in the repeated single-step event with deflectors, whereas in their absence no visible changes were observed. The sequence of discharges affected the frequency of individual sprints and group drifts, particularly evident with deflectors. Additionally, the deflector use increased in the first peak in opposition to the second. Overall, the fish responses were indicative that the low velocity areas (< 0.1$m.s^{-1}$) existing behind the deflectors were favourable under hydropeaking conditions. However, the flow complexity resulting from the presence of deflectors impaired their ability to successfully find them. The integrated approach used in this study provided novel insight considering the responses of cyprinids to hydropeaking in the presence and absence of deflectors as a mitigation measure. This study emphasized the importance of characterizing the hydrodynamic conditions due to hydropeaking, particularly after including the artificial refuges.

Keywords

Hydropeaking; lateral refuges; Iberian barbel; physiology; movement behaviour

3.2.3 Introduction

Hydroelectricity is recognized as a renewable source of energy due its high efficiency, low carbon emissions and production costs (Zarfl et al., 2015). It is predicted to increase, however at a rate that still does not comply with human energetic demand (Zarfl et al., 2015). The consequent large and rapid flow fluctuations in response to the sub daily or daily changes in hydroelectricity demand, termed as hydropeaking (Cushman, 1985; Young et al., 2011), will further aggravate the ecological function of the river ecosystems. Particularly affecting downstream fish communities, the responses to hydropeaking can range from sub-organismal (Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012), to changes in key life-cycle events (Burnett et al., 2014; Casas-Mulet et al., 2015; Kelly et al., 2017a; Korman et al., 2011).

To consider this broad range of responses, different experimental approaches have been adopted to study the effects of hydropeaking. Sub-organism level responses occur by a set of physiological processes after perceiving an environmental disturbance, aiming at restoring the homeostatic state (Pankhurst, 2011). The persistence and severity of the disturbance, and the magnitude of the physiological response, determine the energetic pool available for diel activities (e.g. escaping predators, finding refuge, foraging) that will likely extend to key-life events (e.g. reproduction, survival) and traits (fitness, condition, growth) (Barton, 2002; Pankhurst, 2011). To understand the extent to which the rapid flow fluctuations trigger a stress response, physiological studies have been increasingly conducted over free swimming fish (Krimmer et al., 2011; Taylor et al., 2013, 2012; Taylor and Cooke, 2012), and in simulated conditions in the presence of refuges (Arnekleiv et al., 2004; Flodmark et al., 2002). In these studies the fish movements were not tracked, but the authors attributed the low physiological changes to the presence of available habitats for fish to hide (Flodmark et al., 2002; Krimmer et al., 2011). The presence of refuges was considered beneficial for fish for the highest tested discharges, whereas for the lowest, the stranding probability increased, as well as the probability of incurring in a stress response (Flodmark et al., 2002). The presence of potential cumulative factors other than flow disturbance (e.g. confinement, temperature, nutritional status) (Arnekleiv et al., 2004), and of suitable refuges (Flodmark et al., 2002; Krimmer et al., 2011), increased the difficulty to find cause-effect relationships between flow variability and potential stress responses. To reduce this uncertainty, performing indoor experiments that minimize the confounding effects of external factors, and combine them with adequate physiological indicators, has been encouraged (Flodmark et al., 2002; Young et al., 2011).

Scaling-up to individual, population and even community-level responses, movement behaviour changes are amongst the most studied fish responses to rapid flow fluctuations. In rivers affected by hydropeaking, these changes include small (Krimmer et al., 2011; Scruton et al., 2003; Taylor et al., 2014, 2013) to wide (Boavida et al., 2017; Burnett et al., 2014; De Vocht and Baras, 2003) spatial-scale movements, absent (Jones and Petreman, 2015) and even inconsistent (Scruton et al., 2005). Most studies attributed the absence of visible movements to the river hydromorphology and to the availability of velocity refuges. In this sense, indoor experiments demonstrated to be a valid approach to study smaller scale behavioural changes caused by rapid flow fluctuations. Downstream displacements (Auer et al., 2017), higher search for velocity refuges (Ribi et al., 2014), changes in schooling behaviour (Sloman et al., 2001; Sloman and Armstrong, 2002; Sneddon et al., 2006), and even no evident changes in the movement behaviour (Cocherell et al., 2010; Flodmark et al., 2006) have been reported.

The frequent changes of the water depth, channel width and wetted area, as a consequence of hydropeaking, result in the reduction of suitable habitat and favourable areas for flow refuging (Person et al., 2014). Under these conditions more complex habitats with high availability of refuges should be preferable for fish (Person et al., 2014). However, the created hydraulic instability may increase the risk of stranding, having the opposite effect (Auer et al., 2017; Goettel et al., 2015; Tuhtan et al., 2012; Vanzo et al., 2016). Thus, it is necessary to identify the extent to which habitat complexity creates adverse or favourable flow conditions for fish (Auer et al., 2017; Goettel et al., 2015). An increasing number of studies based on field measurements of riverbed changes (Hauer et al., 2017; Vanzo et al., 2016), fish movement tracking (Boavida et al., 2017; Capra et al., 2017) and habitat suitability curves (Boavida et al., 2015) proposed habitat mitigation measures and operational strategies to hydropeaking consequences based on hydrodynamic models (Boavida et al., 2015; Person et al., 2014). However, their practical applicability has been rarely assessed (Ribi et al., 2014).

The effects of hydropeaking events have been studied mainly for salmonids (Arnekleiv et al., 2004; Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012). In opposition, cyprinids, which are highly representative in most freshwater ecosystems, have been scarcely studied, particularly their movement behaviour and habitat preferences according to the surrounding hydraulic conditions (Vilizzi and Copp, 2005). In this study, an endemic potamodromous cyprinid of the Iberian Peninsula was selected, the Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864) (hereafter *L. bocagei*). This species is widely distributed in the river basins of northern and central Portugal (Lobón-Cerviá and Fernandez-Delgado, 1984; Oliveira et al., 2002). The fingerlings and juveniles are predominantly rheophilic (Martínez-Capel and García de Jalón, 1999; Santos et al., 2017), as well as the spawning adults (Rodriguez-Ruiz et al., 1992). Outside the reproductive season, the adults of *L. bocagei* tend to be limnophilic. To study the

effects of hydropeaking, young adults of *L. bocagei* were used, as they are more adapted to faster flow conditions.

The main objective of this study was to assess the consequences of hydropeaking events and the potential of flow refuges as a mitigation measure. To address this problem, hydropeaking and base-flow experimental trials were conducted in an indoor flume. A multidisciplinary approach was adopted, which combined blood physiology assessment and movement behaviour metrics, together with a hydrodynamic model to characterize the velocity field. Four hypotheses were investigated: 1) In the presence of lateral refuges, simulated hydropeaking events differing in peak-type event trigger different physiological responses and promote changes in the movement behaviour of *L. bocagei*; 2) Under the same hydropeaking event the physiology and movement behaviour of *L. bocagei* are affected by the presence or absence of lateral refuges; 3) In the presence of lateral refuges, peak repetition affects the physiological responses of *L. bocagei*; 4) Different sequences of discharges generate distinct movement behaviour responses.

3.2.4 Methods

Fish sampling and handling

L. bocagei were sampled at the Sorraia River (39.011376 º N, -8.357126 º W), a left tributary of the Tagus River (central Portugal). Generally, the substrate is dominated by sand and pebbles and the vegetation is abundant in macrophytes. The riparian corridor is mainly composed by *Salix* and *Fraxinus* tree species. The sampling site is not affected by hydropeaking which makes it a suitable source of fish that are naïve to hydropeaking events. Fish were sampled once a week in non-consecutive weeks between the 19th of May and the 14th of June 2016 using a low-voltage (400 V) electrofishing gear (Hans Grassl IG-200), according to European norms (European Committee for Standardization – CEN (CEN, 2003) and national guidelines (INAG, 2008). No more than 80 fish were captured per each sampling occasion, resulting in a total of 150 captured fish (mean total length ± SD; 13.6 ± 2.4 cm; mean total weight ± SD; 23.50 ± 12.89 g). After electrofishing, fish were transported in a constantly aerated transport tank (Linn Thermoport 190 l) to the laboratory. *L. bocagei* were equally transferred to two 900 l tanks with ambient temperature and natural photoperiod for a 48-72 hours acclimation period. Each tank was covered with fine meshed garden nets and contained four clay roof tile refuges. The water tank was biologically filtered and permanently aerated. Water quality parameters (mean ± SD) were controlled in a daily basis using a multi parameter probe (YSI 556 MPS) for temperature (21.4 ± 1.1 ^{o}C), pH (6.88 ± 0.22), dissolved oxygen (7.77 ± 0.61 mg.l^{-1}) and conductivity (208.5 ± 47.9 µS.cm^{-1}), and in a

weekly basis using photometry (WTW- Spectroflex 6600) for nitrites (0.02 ± 0.02 mg.l^{-1}) and ammonia (0.001 ± 0.001 mg.l^{-1}). Partial water changes (15%) were performed every other day. Fish were fed with a commercial diet for benthic species every day at night to avoid additional stress caused by food deprivation. Feeding took place only after the acclimation period. The flume water temperature (22.4 ± 1.0 ºC) and dissolved oxygen (7.87 ± 0.61 mg.l^{-1}) were controlled twice a day. The water quality parameters were according to the national legislation for water quality standards to protect and improve the aquatic environment according to water use (Ministry of the Environment, 1998).

Experimental setup

Flume and refuge configuration

The effects of hydropeaking were tested between the 23rd of May and the 17th of June 2016 in an indoor experimental flume located at the Laboratory of Hydraulics (IST, University of Lisbon, Portugal) (Figure 3.10). The flume, with a rectangular cross-section, was built on a steel frame with glass panels on both sides. An upstream reservoir controlled by a plane gate enabled rapid discharge variations, and a downstream flap gate controlled the water level. The maximum discharge was set to 60 l.s^{-1}. The usable area for fish was limited by two perforated metallic panels creating a 6.5 m long-reach (Figure 3.10).

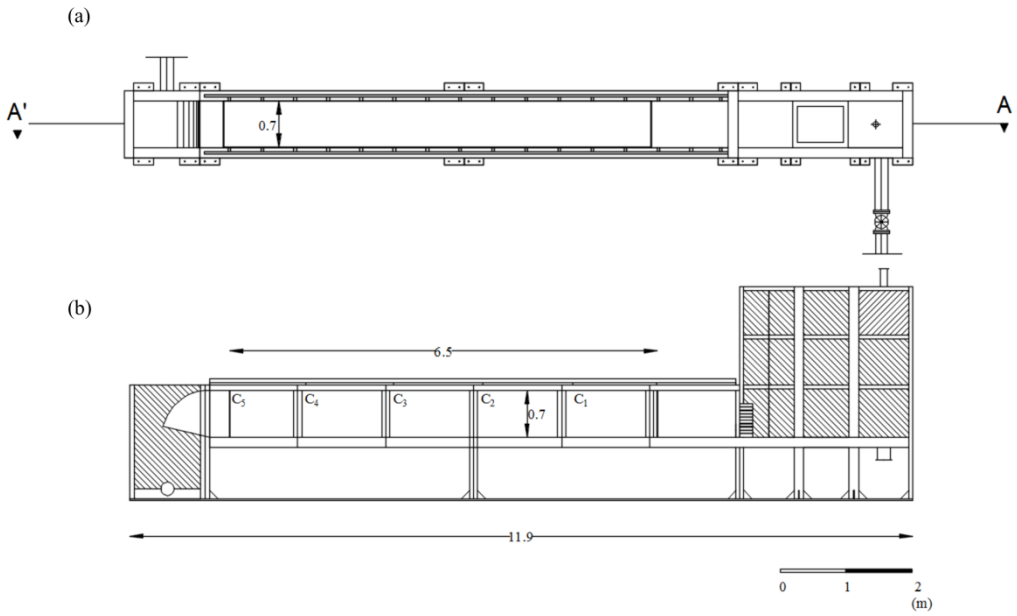

Figure 3.10 Top (a) and lateral (b) view of the indoor experimental flume. The dimensions (m) and behaviour observation areas (C1 to C5) are represented.

To mimic lateral refuges of a river channel, three PVC flashboards (0.30 m x 0.76 m x 0.015 m), herein termed as deflectors (D1-D3), were installed in the PVC false bottom of the flume (Figure 3.11). The three deflectors occupied 1/3 of the flume width assured by a 45º opening angle (Figure 3.11). This distance allowed the fish to swim freely and to fully exhibit their swimming behaviour. This selected configuration simulated a river segment with a heterogeneous bank which assured velocity refuges, in opposition to a homogenous bank. This configuration was selected to check whether structural mitigation measures proposed for only one bank, maintaining the other bank undisturbed, provided sufficient refuge and were able to mitigate the consequences of hydropeaking.

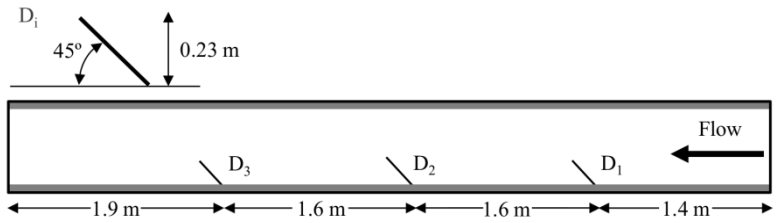

Figure 3.11 Representation of the top view of the flume with the refuge (deflector) configuration tested. The position of the deflectors in the flume (m) and a detail of their dimensions (m) are shown. (D_i, i = 1 to 3).

Hydropeaking events and hydraulic characterization

The tested hydropeaking events mimicked the operation of a Portuguese hydropower plant (Pragana et al., 2017). Given the three discharges in test, i.e. two peak discharges (40 $l.s^{-1}$ and 60 $l.s^{-1}$) and a base-flow discharge (7 $l.s^{-1}$), the flow ratios, defined as the maximum flow divided by the minimum flow (Q_{Peak}/Q_{Base}) (Sauterleute and Charmasson, 2014), were 5.7 and 8.6 respectively, which are considered high (Harby and Noack, 2013; Sauterleute and Charmasson, 2014). A hydropeaking event was defined as a sequence of discharges, differing in peak-type event and peak frequency, where the time of exposure to each discharge was at least 20 minutes (Figure 3.12). The study was divided into two main experiments. In the first experiment (E1), two hydropeaking (HP) events were tested, differing in the sequence of discharges (peak-type event) over time, and in the flow ratios (Figure 3.12, Table 3.6).

For this experiment the flume was permanently equipped with deflectors (D). The two hydropeaking events are described as follows: (1) E1HP1D consisted of a single-step event where *L. bocagei* were exposed to a continuous 60 $l.s^{-1}$ peak discharge for 40 minutes; (2) E1HP2D consisted of a two-steps event, where *L. bocagei* were exposed to 40 $l.s^{-1}$ during the first 20 minutes, and to 60 $l.s^{-1}$ during the last 20 minutes of the event (Figure 3.12, Table 3.6).

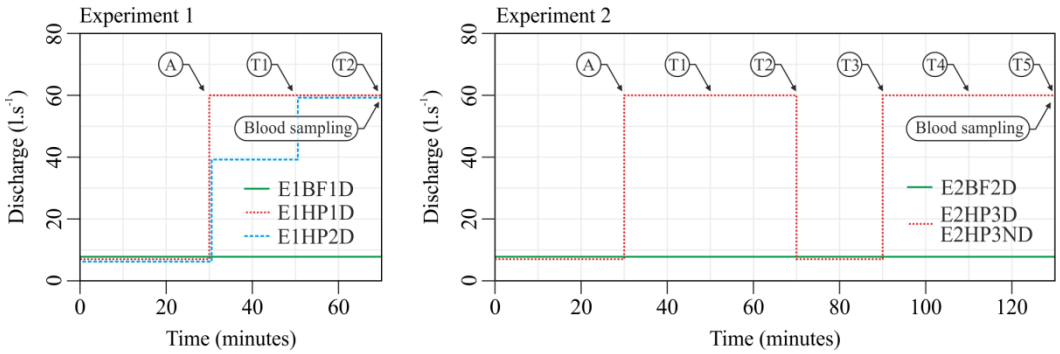

Figure 3.12 Flow events tested in experiments 1 and 2. In experiment 1 two hydropeaking events were tested in the presence of deflectors: E1HP1D, consisting of a single-step event, and E1HP2D, consisting of a two-steps event, and a base-flow event (E1BF1D). In experiment 2, one hydropeaking event was tested for the presence and absence of deflectors respectively: E2HP3D and E2HP3ND, consisting of a repeated single-step event, and a base-flow event (E2BF2D). (A) Acclimation period. (T1 to T5) correspond to the periods of time of movement behaviour observation. (Blood sampling) indicates the time when blood was collected for physiological analysis.

These events were compared with a base-flow event (E1BF1D), where *L. bocagei* were subjected to a continuous 7 $l.s^{-1}$ discharge for 40 minutes (Figure 3.12, Table 3.6). In the second experiment (E2) one hydropeaking event was tested for two different deflector configurations: presence and absence. The simulated hydropeaking event consisted of a repeated single-step event, where *L. bocagei* were subjected to two peaks of 60 $l.s^{-1}$ (40 minutes each peak) interpolated with the base-flow of 7$l.s^{-1}$ (20 minutes) (Figure 3.12, Table 3.6). This event was tested with deflectors (E2HP3D) and without deflectors (E2HP3ND) (Table 3.6). The base-flow event consisted of a continuous 7 $l.s^{-1}$ discharge event (100 minutes) with deflectors (E2BF2D) (Figure 3.12, Table 3.6).

Table 3.6 Flow events tested. The events changed according to: the tested discharges (base-flow, 7 $l.s^{-1}$; peak-flow, 40 $l.s^{-1}$ and 60 $l.s^{-1}$), the sequences of discharges, peak-type event, peak frequency, presence of a base-flow, and deflector presence or absence. Before each hydropeaking event there was an acclimation period where *L. bocagei* were subjected to a 7 $l.s^{-1}$ discharge for 30 minutes.

Experiment	Event	Discharge sequences ($l.s^{-1}$)*	Time sequences (minutes)*	Peak-type event	Peak repetition	Base-flow	Deflector
E1	E1HP1D	60	40	Single-step			X
	E1HP2D	40-60	20-20	Two-steps			X
	E1BF1D	7	40	no step		X	X
E2	E2HP3D	60-7-60	40-20-40	Single-step	X	X	X
	E2HP3ND	60-7-60	40-20-40	Two-steps	X	X	
	E2BF2D	7	100	no step		X	X

*excluding the 30 minutes acclimation

Before each event, *L. bocagei* were acclimating for 30 minutes under 7 $l.s^{-1}$. During this period, the flume upstream gate was maintained open with a 10º angle, and the downstream gate with a 76º angle, which was fixed throughout all experiments. To simulate a hydropeaking event, the upstream gate was closed

while filling up the flume reservoir until attaining the tested peak flow by manually controlling the discharge. Afterwards, the upstream gate was rapidly opened to 10º while releasing the tested peak flow and reaching the permanent regime. The mean time (± SD) for this procedure (up-ramping phase) for the maximum tested discharge was 28.12 ± 1.86', corresponding to an up-ramping rate of 0.55 $cm.s^{-1}$ with a total increase of 15.7 cm in the water depth until reaching permanent regime.

Each hydropeaking and base-flow event tested comprised a school of five *L. bocagei* and was replicated five times. Each fish was tested only once. Bigger groups of *L. bocagei* likely occur in nature, particularly during the reproductive season, however, a school of five fish was selected to optimize the observation of fish movement behaviour in the flume, to reproduce representative group behaviour (Weihs, 1973), and to reduce the number of fish to be collected from the wild.

A detailed flow velocity characterization of the flume was previously conducted using an Acoustic Doppler Velocimeter (ADV), the Nortek-AS Vectrino 10 MHz, with Vectrino Plus firmware. Those velocity measurements were used to calibrate the FLOW-3D® hydrodynamic model (Flow Science Inc., 2012). This model was used as a complementary tool to better understand the movement behaviour patterns of *L. bocagei* according to the results of the frequency of deflector use and swimming activity in the flume.

Fish responses

Physiological responses

The levels of blood glucose and lactate were assessed to find physiological changes as potential responses to hydropeaking as a stressor, and to the presence of deflectors. Changes in glucose and lactate levels have been widely used as secondary physiological indicators of stress to flow variability (Costa et al., 2017). Increases in the glucose and lactate levels are usually directly associated with primary responses to stress (Pankhurst, 2011), and the lactate levels will likely increase when aerobic swimming is no longer sufficient to maintain sustained swimming, resulting in exhaustion (Wendelaar Bonga, 1997). Hence, both physiological responses are reliable proxies of a stress response to flow variability. After each event, each fish was dip-netted from the flume and transferred to a recipient with permanently oxygenated water and immediately placed in a v-shaped plastic trough in a supine position. Blood samples (0.1-0.5 ml) were collected via caudal puncture using 23 G or 25 G pre-heparinized needles within 3 minutes of capture. Laboratory studies have demonstrated that capture-related stress will not significantly influence primary stress responses (e.g. cortisol) if the sample is taken quickly (Sumpter, 1997). The glucose and lactate levels were immediately measured using the portable

meters Accu-check Aviva (Roche) and Lactate Plus (Nova Biomedical UK) respectively. Lactate and glucose portable meters have been confirmed to provide valid results (Beecham et al., 2006; Stoot et al., 2014; Tanner et al., 2010).

Movement behaviour

The behaviour metrics were divided into two categories: deflector use and swimming activity in the flume. The movement behaviour frequency was quantified as the number of occurrences (absolute frequency) of each behaviour pattern. It was registered during periods of 20 minutes, in order to verify the effect of discharge sequence over time. In the first experiment (E1) it was registered during two slots of 20 minutes, T1 and T2 (Figure 3.12). In the second experiment (E2) it was registered during five slots of 20 minutes, T1 to T5 (Figure 3.12). Although E1 and E2 had equal periods where *L. bocagei* were subjected to the same conditions, the movement behaviour metrics were analysed separately to allow further discussion together with the physiological results that were obtained after each event of each experiment. Otherwise the results from the movement behaviour metrics would be overestimated and the discussion would be biased. The deflector use was counted for each deflector (D1-D3), and registered as individual or group (2 to 5 fish) use (Iuse or Guse) respectively.

To evaluate the potential effect of hydropeaking events on the movement behaviour of *L. bocagei* the selected metrics represented immediate movement responses. Thus, the selected swimming activity metrics were fish sprints, defined as a sustained swimming activity lasting a few seconds, characterized by several tail beats; and fish drifts defined as voluntary or involuntary downstream fish displacements associated to drag. They were counted for five observation areas (C1 to C5, Figure 3.10) and their frequencies were registered for each individual (Isprint and Idrift) and group (Gsprint and Gdrift) (2 to 5 fish). A behaviour occurrence was assigned to a specific area (C1 to C5), only if it had started there. Each individual or group were considered a sampling unit and any individual behaviour was only registered if performed by one fish independently of the group. The fish movement behaviour was visually assessed by two observers who registered the occurrences on an ethogram sheet. Observer one monitored C1 to C3, and observer two monitored C4 to C5 (Figure 3.10). Each observer recorded all behaviour metrics. During the acclimation period (30 minutes) there were no behavioural observations. To avoid differences in behaviour observation results, the two observers assessed the same area during pilot tests, and the movement behaviour results were considered satisfactory when a good agreement between the movement behaviour results obtained by the observers was detected.

Data analysis

A non-parametric statistical analysis was performed to investigate whether: 1) the blood physiology of *L. bocagei* differed between the hydropeaking and the base-flow events in the presence of deflectors (E1); 2) under the same hydropeaking event, the blood physiology differed between the presence and absence of deflectors, and in comparison with the base-flow event with deflectors (E2); and 3) in the presence of deflectors, peak frequency promoted physiological adjustments in *L. bocagei* (E1HP1D *vs.* E2HP3D). A preliminary data exploration was conducted to check for outliers in the blood physiology levels for each replicate (n=5) by means of boxplot analysis. If outliers were detected, the glucose and lactate levels were interpreted to verify if they corresponded to real outliers. This avoided losing individual information that would not stand out if the raw data were transformed or mean values were used to follow the assumptions required to apply parametric tests. Afterwards, to verify if there were any significant differences in the levels of glucose and lactate of *L. bocagei* between the events of each experiment (E1 or E2) a non-parametric Kruskal-Wallis test was applied, with a Nemenyi post-hoc test for pairwise contrasts (Pohlert, 2015). In case there were ties between mean rank sums, the Chi-square approach was used (Pohlert, 2015). The glucose and lactate levels observed in E1HP1D (one 60 $l.s^{-1}$ peak, 40 minutes, with deflectors) and E2HP3D (two 60 $l.s^{-1}$ peaks, 100 minutes, with deflectors) were compared by the Wilcoxon Rank sum test. These statistical analyses were performed for α=0.05 using R version 3.3.2 (R Core Team, 2017).

A Mixed-Design ('Split-Plot') ANOVA was conducted to evaluate the effects of: (1) the hydropeaking event and the sequence of discharges in the presence of deflectors (E1); and 2) the hydropeaking event and peak frequency in the presence and absence of deflectors (E2), on the movement behaviour of *L. bocagei*. As the movement behaviour was registered during every 20 minutes (T1 and T2 for E1, and T1 to T5 for E2), this test analysed the differences in the movement behaviour frequencies in a sequential manner over time. Consequently, it was possible to investigate the potential cumulative effect of the peak discharges over time. To use this test, the normality assumption of the frequency of deflector use and swimming activity for each time-period was previously verified. Likewise, the assumption of the homogeneity of variances was previously verified by the Box's M test for E1 (Box, 1949) and Mauchly's test of sphericity of the variance-covariance matrix for E2 (Mauchly, 1940). The Greenhouse-Geisser correction was used when the homogeneity of variance was not verified (Greenhouse and Geisser, 1959). The analysis was conducted separately for deflector use and swimming activity for the events of both experiments for α=0.05 using PASW statistics 18 ("PASW Statistics for Windows," 2009).

3.2.5 Results

Hydraulics

The hydrodynamic model developed with FLOW-3D® showed that the minimum flow velocities occurred in the downstream area of the deflectors for both the base-flow (7 $l.s^{-1}$) and the highest peak discharge (60 $l.s^{-1}$) tested (Figure 3.13). The area with near zero velocities was larger for the 7 $l.s^{-1}$ and occurred mainly along the left side of the flume where the deflectors were installed. Along the opposite side, the flow velocity was always lower than 0.17 $m.s^{-1}$ (Figure 3.13).

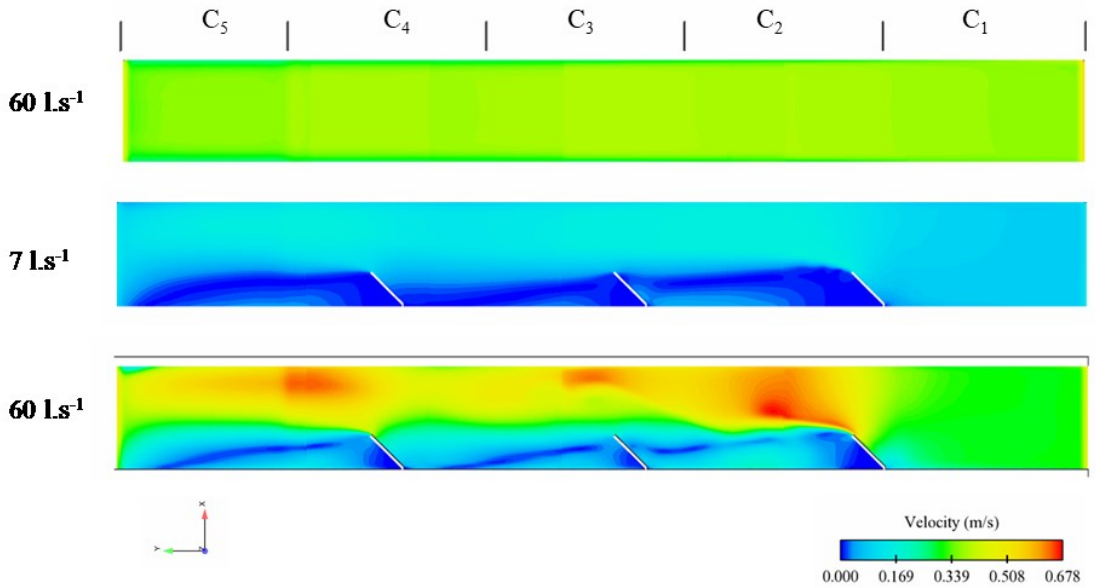

Figure 3.13 Mean flow velocity fields for 7 and 60 $l.s^{-1}$ obtained with FLOW-3D®. The numerical models were calibrated according to previous velocity field measurements obtained with an ADV for the same flow discharges. C_1 to C_5 correspond to the observation areas.

For 60 $l.s^{-1}$ the flow velocity field was more heterogeneous in the presence of deflectors than without them, as expectable (Figure 3.13). Mean velocities were higher in the upstream area of the flume, particularly in the vicinity of D1 and progressed through the right length of the flume, where there were no refuges (Figure 3.13).

Physiological responses

Considering E1, the mean (± SEM) levels of blood glucose in *L. bocagei* were 47.5±4.19 $mg.l^{-1}$, 54.5±3.10 $mg.l^{-1}$, and 55.6±4.42 $mg.l^{-1}$ for E1HP1D, E1HP2D and E1BF1D respectively (Figure 3.14).

Although the glucose levels were higher in E1BF1D, these differences were not statistically significant (χ^2 (2) = 4.035, P = 0.133). The mean (± SEM) levels of blood lactate in *L. bocagei* were 1.74±0.25 mM, 2.01±0.20 mM, and 2.23±0.36 mM for E1BF1D, E1HP1D and E1HP2D respectively (Figure 3.14). The levels of this indicator for exhaustive exercise did not differ significantly between those three events (χ^2 (2) = 1.043, P = 0.594). In E2, the mean (± SEM) glucose levels in *L. bocagei* were 45.7±4.96 mg.l^{-1}, 49.6±3.03 mg.l^{-1}, and 60.8±3.94 mg.l^{-1} for E2HP3ND, E2BF2D and E2HP3D respectively (Figure 3.14).

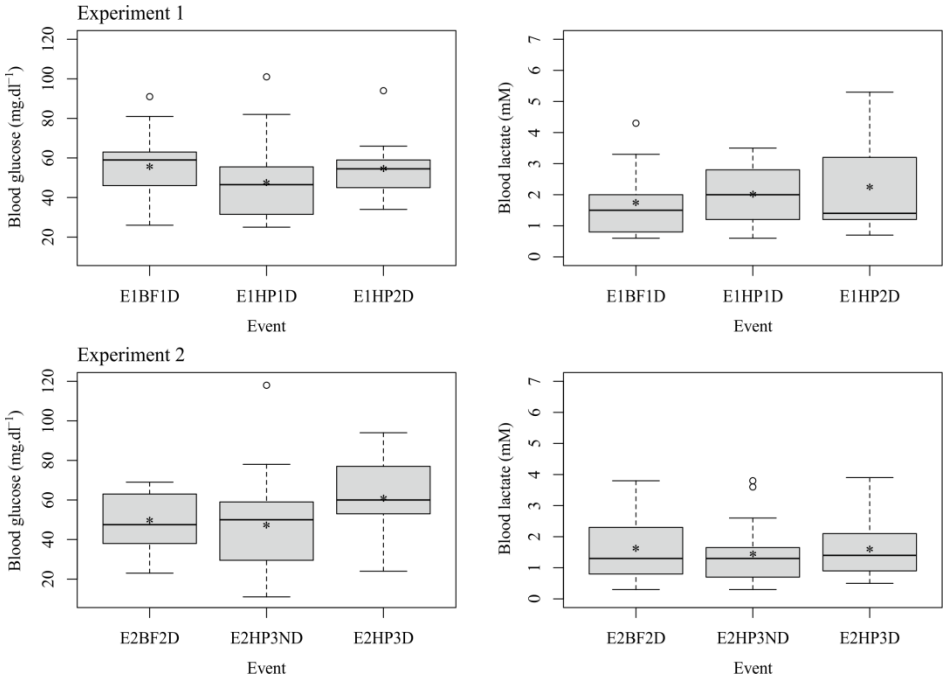

Figure 3.14 Boxplots with the variation of blood glucose (mg.dl^{-1}) and lactate (mM) levels for *L. bocagei* subjected to the events from experiments 1 and 2. Experiment 1: E1HP1D, E1HP2D and E1BF1D correspond to the single-step, two-steps and base-flow events tested for the presence of deflectors respectively; Experiment 2: E2HP3D, E2HP3ND and E2BF2D correspond to the repeated single-step event in the presence, absence and the base-flow events respectively. The asterisk corresponds to the mean value of the physiological indicator of each event.

The glucose levels differed significantly between the three events (χ^2 (2) = 10.237, P = 0.006) and were significantly higher for *L. bocagei* subjected to the repeated single-step event in the presence of deflectors (E2HP3D) in comparison with the same event without deflectors (E2HP3ND) (χ^2 = 9.619, P = 0.008) (Figure 3.14). The mean (± SEM) lactate levels in *L. bocagei* were 1.43±0.19 mM, 1.59±0.18 mM, and 1.62±0.20 mM for E2HP3ND, E2HP3D and E2BF2D respectively (Figure 3.14). The lactate levels did not differ significantly between the three events of E2 (χ^2 (2) = 0.683, P = 0.710). Considering the effect of peak repetition, the glucose levels of *L. bocagei* that were subjected to the repeated single-step event (E2HP3D) were significantly higher in comparison with the single-step event without repetition (E1HP1D) (W = 120, P = 0.006). The lactate levels did not differ between these two events (W = 285, P = 0.1839).

Movement behaviour

Experiment 1 – Flow event effect

The mean frequency of Iuse differed significantly between E1HP1D, E1HP2D and E1BF1D ($F(2,10) = 5.332$; $P = 0.027$) (Figure 3.15 a). Pairwise comparisons evidenced that it was higher in E1HP1D in comparison with E1BF1D ($P = 0.035$).

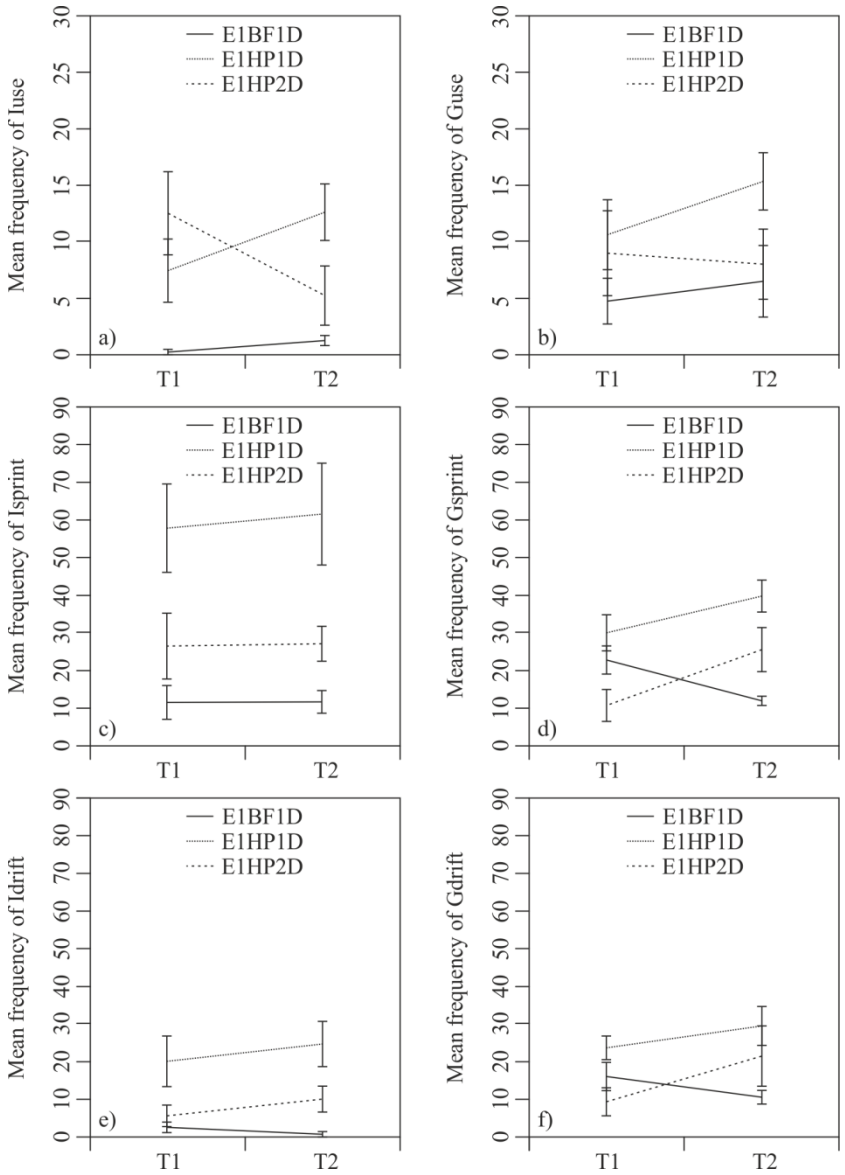

Figure 3.15 Mean frequency (± SEM) of deflector use (Iuse and Guse) and flume swimming activity (Isprint, Gsprint, Idrift and Gdrift) of *L. bocagei* in experiment 1. E1HP1D, E1HP2D and E1BF1D correspond to the single-step, two-steps and base-flow events tested for the presence of deflectors respectively. T1 and T2 correspond to the periods of time of observation of movement behaviour.

Between these two events, in 95% of the individual deflector use, *L. bocagei* subjected to E1HP1D approached the deflectors 1-18 times more in average than in E1BF1D. Although the mean frequency of Guse was higher in both peak events, i.e. E1HP1D and E1HP2D, in comparison with E1BF1D (Figure 3.15 b), this difference was not statistically significant ($F(2,10) = 2.988$; $P = 0.096$). The frequency of Iuse and Guse followed a similar trend between the two slots of 20 minutes (T1-T2) for the three events ($F(2,10) = 3.179$; $P = 0.085$; $F(2,10) = 0.331$; $P = 0.726$ respectively). Thus, the differences on the frequency of deflector use observed between hydropeaking and the base-flow event were not reflected by the time that the fish were subjected to a certain discharge. Even though, in the single-step event (E1HP1D) the mean frequency of Iuse and Guse was more pronounced during the last 20 minutes (T2) of exposure to 60 $l.s^{-1}$, whereas in the two-steps event (E1HP2D) it was more pronounced in the first 20 minutes (T1), where fish were exposed to 40 $l.s^{-1}$ (Figure 3.15 a-b).

The mean frequency of swimming activity differed significantly between events (E1HP1D, E1HP2D and E1BF1D) for Isprint ($F(2,10) = 12.197$; $P = 0.002$) and Gsprint ($F(2,10) = 5.364$; $P = 0.026$), and Idrift ($F(2,10) = 4.934$; $P = 0.032$) (Figure 3.15 c-e). Pairwise comparisons evidenced that the frequency of Isprint and Idrift were significantly higher in E1HP1D than in E1BF1D ($P = 0.002$ and $P = 0.039$ respectively) (Figure 3.15 c,e). Isprint was also higher in E1HP1D in comparison with E1HP2D ($P = 0.025$) (Figure 3.15 c). Gdrift remained similar between the three events ($F(2,10) = 2.424$; $P = 0.139$) (Figure 3.15 f).The differences in the swimming activity observed between the three events were not reflected by the time that the fish were subjected to a certain discharge. Isprint ($F(2,10) = 0.035$; $P = 0.966$), Idrift ($F(2,10) = 1.037$; $P = 0.390$) and Gdrift ($F(2,10) = 3.330$; $P = 0.078$) followed the same trend in T1 and T2 (Figure 3.15 c,e,f). On the other hand, Gsprint followed an opposing trend between T1 and T2 ($F(2,10) = 9.068$; $P = 0.006$). This can be observed in the single-peak (E1HP1D) and in the two-peak (E1HP2D) events in comparison with the base-flow event (E1BF1D) (Figure 3.15 d).

Experiment 2 – Deflector effect

The mean frequency of Iuse ($F(1,8) = 22.285$; $P = 0.001$) and Guse ($F(1,8) = 33.627$; $P < 0.001$) differed significantly between the two-steps event with deflectors (E2HP3D) in comparison with the respective base-flow event (E2BF2D) (Figure 3.16 a-b). *L. bocagei* used the deflectors individually and in groups more frequently in the first (Figure 3.16 a-b). Between these two events, in 95% of the approaches, *L. bocagei* that were subjected to E2HP3D used the deflectors individually 7-17 and in group 8-19 more in average in comparison with E2BF2D. Furthermore, the sequence of discharges over time affected Guse ($F(4,32) = 3.345$; $P = 0.021$), but not Iuse ($F(1.717,13.735) = 3.528$; $P = 0.064$). This effect was particularly evident between T2 and T3 and T3 and T4 in E2HP3D (Figure 3.16 b). As in

E2BF2D the discharge was constant from T1 to T5, the pattern of deflector use remained similar between the five slots of 20 minutes (Figure 3.16 a-b).

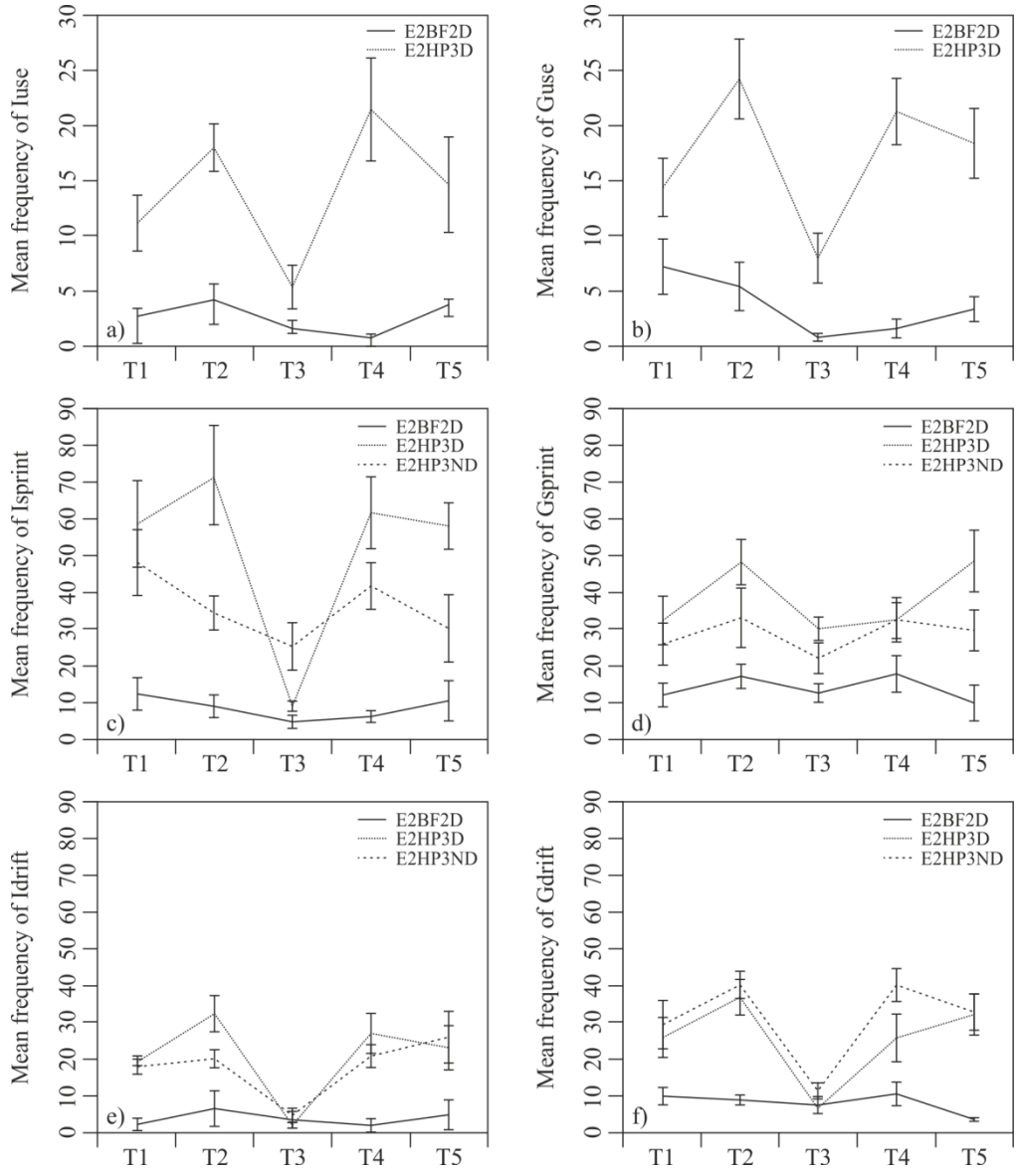

Figure 3.16 Mean frequency (± SEM) of deflector use (Iuse and Guse) and flume swimming activity (Isprint, Gsprint, Idrift and Gdrift) of *L. bocagei* in Experiment 2. E2HP3D and E2HP3ND: repeated single-step event in the presence and absence of deflectors respectively. E2BF2D: base-flow event in the presence of deflectors. T1 to T5: periods of time of observation of movement behaviour.

The mean frequency of flume swimming activity differed significantly between E2HP3D, E2HP3ND and E2BF2D for Isprint ($F(2,12) = 15.290$; $P < 0.001$) and Gsprint ($F(2,12) = 6.373$; $P = 0.013$), and for Idrift ($F(2,12) = 13.977$; $P = 0.001$) and Gdrift ($F(2,12) = 16.652$; $P < 0.001$) (Figure 3.16 c-f). All swimming activity metrics were significantly higher in E2HP3D in comparison with E2BF2D (Isprint, $P < 0.001$;

Gsprint, P = 0.012; Idrift, P = 0.001; Gdrift, P = 0.004). Additionally, Isprint, Idrift and Gdrift were significantly higher in E2HP3D in comparison with E2BF2D (P = 0.014, P = 0.004, and P < 0.001 respectively). Furthermore, the sequence of discharges over time (T1 to T5) affected Isprint (F(8,48) = 3.770; P = 0.002) and Gdrift (F(8,48) = 3.132; P = 0.006), but not Gsprint (F8,48) = 1.693; P = 0.125) and Idrift (F(4.948,29.686) = 2.489; P = 0.054). This effect was evident in E2HP3D and E2HP3ND in comparison with the E2BF2D (Figure 3.16 c-f). In this event swimming activity metrics remained constant during the five 20-minute slots (Figure 3.16 c-f).

3.2.6 Discussion

The consequences of the simulated hydropeaking events and the potential of lateral deflectors as flow refuges to hydropeaking consequences were investigated. A multidisciplinary approach combined physiological and behavioural responses with a characterization of the flow velocity field with hydrodynamic models. The hydropeaking events and the refuge configurations tested resulted in distinct fish responses (Figure 3.15-Figure 3.16). In the presence of deflectors, both the single-step (E1HP1D) and the two-steps (E1HP2D) events failed to elicit changes in the glucose and lactate levels of *L. bocagei* (first hypothesis). However, in the presence of deflectors, peak-repetition (E2HP3D) resulted in a glucose increase in comparison with the repeated single-step peak event without deflectors (E2HP3ND) (second hypothesis), and with the single-step peak event with deflectors (E1HP1D) (third hypothesis) (Figure 3.14). The evident differences in the movement behaviour responses between peak-type event in the presence of deflectors (Figure 3.15), presence and absence of deflectors for the same hydropeaking event (Figure 3.16), and discharge sequence over time (Figure 3.15-Figure 3.16), corroborated the first, second and the fourth hypotheses respectively.

In experiment 1, the relatively stable physiology levels between *L. bocagei* subjected to the two tested hydropeaking events indicate that a 5.7 followed by an 8.6 flow ratio (E1HP2D), and a continuous 8.6 flow ratio (E1HP1D) were not severe enough to promote physiological changes. The stressor severity, its combination with other external stimuli, and the timespan between stimulus perception to blood collection may result in different and even contrasting stress responses over time (Costa et al., 2017; Pankhurst, 2011). For example, Arnekleiv et al., 2004 detected peak levels of blood glucose in brown trout immediately after electrofishing, levelled-off after a 72 hour acclimation period, increased again after dewatering (simulating the down-ramping stage of a hydropeaking event), and sustained for at least 24 hours after the dewatering stage. However, for the same species, there was no change in the blood glucose levels during dewatering (Flodmark et al., 2002). In the present study, although the two-steps event, characterized by a transition from a lower to a higher peak, could represent a potential

operational mitigation measure to a same duration single-step event, there is no physiological evidence that it would be beneficial to fish.

The most adverse impacts on fish result from the combined alterations of various flow parameters (Harby and Noack, 2013) during hydropower plant operation. In E2HP3D, the inflicted flow variability and the presence of the lateral refuges created a complex flow environment, particularly evident in the vicinity of D1 and on the right side of the flume (Figure 3.13), which seemed to be disadvantageous for *L. bocagei*. The high velocities (> 0.6 $m.s^{-1}$) observed in the vicinity of the deflectors may have reduced the ability of *L. bocagei* to find the lower velocity areas (< 0.1 $m.s^{-1}$) existing in the downstream area of the deflectors (Figure 3.13). The unstable hydraulic conditions, together with peak repetition, resulted in physiological adjustments, indicating that a higher peak frequency, together with sustaining the peak duration to an 8.6 flow ratio, triggered a physiological response. Conversely, *L. bocagei* that were subjected to E2HP3ND had significantly lower glucose levels (Figure 3.14). This indicates that the velocity stability (≈ 0.35 $m.s^{-1}$) resulting from the absence of deflectors prevented any potential physiological adjustments (Figure 3.13).

The hydropeaking conditions tested in both experiments failed to elicit significant changes in the levels of blood lactate. Either the simulated hydropeaking events were not significantly severe to promote burst swimming or the timespan until blood collection was not sufficient to cause such an elevation (e.g. Arnekleiv et al., 2004). Additionally, the smaller size of the young adults, which is related with higher fatigue speeds, possibly contributed to an optimized swimming performance (Mateus et al., 2008). Milder stressors usually result in later and less evident peak responses (Barton et al., 1980), thus the trade-off between upstream movement and flow avoidance by flow refuging assured by the presence of deflectors, could have contributed to the maintenance of low lactate levels in *L. bocagei*. Similarly, Flodmark et al., 2002 and Krimmer et al., 2011 suggested that the less pronounced stress response in brook trout and brown trout in hydropeaking rivers and in simulated down-ramping indoor experiments respectively, could be explained by the presence of refuges. In nature, fish are affected by a diverse array of adverse stimuli that can promote morphological and behavioural adaptations as a result from trade-offs between avoiding the stressor and maintaining diel activities (Sunardi et al., 2005). However, prolonging the flow variability disturbance would likely decrease the ability of fish to develop mechanisms to compensate and adapt (Flodmark et al., 2002).

As for movement behaviour there were marked differences between hydropeaking and base-flow events in the presence of deflectors (Figure 3.15) and between configurations (Figure 3.16). In addition, the individual and group behaviour dynamics also differed significantly (Figure 3.15Figure 3.16). In the

first experiment, the marked individual deflector use in the first 20 minutes of E1HP2D (Figure 3.15 a) could suggest that a two-steps peak event, characterized by a lower peak discharge followed by a higher one, favoured *L. bocagei* to individually find a velocity refuge. Even if the presence of deflectors created a complex flow environment, the velocity field existing under 40 $l.s^{-1}$, probably favoured positive rheotaxis and their ability to effectively find the refuges alone. It is also documented that the smaller size of young adults favours rheophilic behaviour at this life-stage (Rodriguez-Ruiz and Granado-Lorencio, 1992). Fish usually tend to avoid demanding hydraulic conditions, choosing low flow areas for flow refuging (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012; Vehanen et al., 2000). Similarly, in studies where fish were subjected to pulsed flows with different levels of intensity, the swimming activity was always more pronounced during the flow disturbance (Chun et al., 2011; Kelly et al., 2017b; Vehanen et al., 2000), and to avoid unstable hydraulic conditions fish hid in available rocky substrates (Chun et al., 2011), and lateral refuges (Ribi et al., 2014). The transition to a higher peak discharge in this event resulted in a marked decrease in the individual deflector use (Figure 3.15 a), possibly explained by the complex flow conditions created during the discharge transition, reducing the *L. bocagei* ability to successfully find the deflectors alone. Both individual and group deflector use were more pronounced during the last 20 minutes of E1HP1D, indicating that the velocity field existing under 60 $l.s^{-1}$ and that maintaining a stable discharge stimulus (single-step event), promoted deflector use for flow refuging.

In the second experiment, where the presence and absence of deflectors were tested for a repeated single-step event, *L. bocagei* used the deflectors more frequently under the 60 $l.s^{-1}$ flow peak (Figure 3.16 a-b). In addition, the frequency of Guse differed during each 20-minute of behavioural observations. In E2HP3D the individual and group deflector use followed an identical trend over the five 20-minute slots (Figure 3.16 a-b). Notably, for each 20 minutes, and in comparison with E2BF2D, the differences were more pronounced for the frequency of Guse, particularly in T2 and T5 (Figure 3.16 b). The higher frequency of Guse registered in T2, may indicate that *L. bocagei* were able to cope with the unstable hydraulic conditions and to benefit from group behaviour to successfully find the deflectors for flow refuging. The lower frequency of Guse observed particularly in T5 indicates that their ability to cope with the unstable hydraulic conditions may be reduced. This interpretation is corroborated by the increased glucose levels, suggesting a physiological adjustment. Grounded in these interpretations a longer period of time of exposure to a base-flow discharge is recommended between two peak discharges.

For both experiments, the swimming activity was more pronounced in the hydropeaking than in the base-flow events (Figure 3.15Figure 3.16). Visible increases in the swimming activity of fish have been

reported not only in indoor and outdoor flumes (Auer et al., 2017; Ribi et al., 2014), but also in rivers affected by hydropeaking (De Vocht and Baras, 2003; Kelly et al., 2017). In this study, the frequency of Isprint was particularly marked in E1HP1D and E2HP3D (Figure 3.15Figure 3.16). In these two events, *L. bocagei* were exposed to the highest and the longest peak discharge and to a heterogeneous flow environment assured by the presence of deflectors. Additionally, in E2HP3D *L. bocagei* were also subjected to a repeated peak. The evident increase in the frequency of Isprint in E1HP1D and the highest frequency of both Isprint and Gsprint during the last 20 minutes of both hydropeaking events tested in the first experiment (Figure 3.15 c-d) indicated that positive rheotaxis is intensified under the highest peak discharges. However, it also suggests that the adverse hydraulic conditions created promote some degree of group behaviour disruption.

In the second experiment the frequency of Isprint was affected by the sequence of peak and base-flows (Figure 3.16 c). This effect was more visible in the presence of deflectors (E2HP3D). The concomitant glucose adjustments in this event suggest that the ability of *L. bocagei* to cope with a twofold peak frequency could be reduced, probably due to the higher energetic costs associated to sprinting and station holding, which is a function of the fish drag multiplied by the water velocity (Webb, 1988). The same effect was verified for Gdrift and was more evident in the absence of deflectors (E2HP3ND) (Figure 3.16 f), strengthening the interpretation that the sequence of peak and base-flows over time could also influence the group dynamics of *L. bocagei* .The simplification of the velocity field due to the absence of deflectors favoured group drifts, either they were voluntary or not. Group drifting may represent an adaptive compensation mechanism (Flodmark et al., 2002) for *L. bocagei* to recover from the effort required to move upstream by sprinting, which implies an increase in the tail beat frequency (e.g. Clark et al., 2010), and station holding. With complex flow patterns, fish in groups develop mechanisms to cope with the flow disturbance by finding low velocity areas and optimizing fluid-body interactions (Liao, 2007). By exploiting relatively regular recirculating conditions, fish in groups usually benefit from the increased tail beat frequency of the leading fish, which could be translated in a reduction of total swimming costs (Enders et al., 2005). However, in a long term, the reduced availability of velocity refuges will likely decrease the fish ability to successfully cope with highly fluctuating flow environments. In this study, the high frequency of Isprint, particularly evident in E2HP3D (Figure 3.16 c) may indicate that higher velocities (> 0.6 $m.s^{-1}$) (Figure 3.13) and the presence of deflectors, created a flow environment that determined the group dynamics of *L. bocagei*. Likewise, the social hierarchy of brown trout was affected by simulated spates in artificial tanks: the less dominant fish experienced reduced growth rates (Sloman et al., 2001) and the advantages of social dominance were lost (Sloman et al., 2002).

In nature, even a highly regulated river is unlikely bare and the changes in the river hydromorphology due to hydropeaking, still create opportunities for fish to find refuges. The extreme simplification of the velocity field in the absence of deflectors implied that, under the same hydropeaking event, the hydraulic conditions were less adverse (Figure 3.13). Complex habitats create contrasting environmental conditions: more available refuges to hide and save energy, and, in opposition, unstable hydraulic conditions created by unpredictable changes in the velocity fields.

3.2.7 Prospects and applicability

Recognizing the limitations of indoor experimental studies, the integrated use of behaviour quantification tools (blood physiology and movement behaviour frequency) with the characterization of the flow environment using hydrodynamic models, strengthened the interpretation of the fish responses. The presence of deflectors increased the flume heterogeneity while providing low velocity areas. However, under rapid flow fluctuations, that heterogeneity may represent an additional constraint for *L. bocagei*, by reducing their ability to find refuge behind the deflectors. In this study, the flow heterogeneity resultant from the rapid flow fluctuations and the presence of the deflectors generated distinct behavioural responses. In natural rivers affected by hydropeaking, fish behaviour is also affected in distinct ways. In this sense, before conceptualizing potential velocity refuges to implement in natural conditions, it is necessary to characterize the rapid flow fluctuations, and the extent to water depth, velocity fields and wetted profile change (Schmutz et al., 2015). Afterwards, the proposed velocity refuges (deflectors) may be tested using hydrodynamic models to understand whether the added habitat heterogeneity provides velocity refuging areas, or creates unstable hydraulic conditions for fish (Auer et al., 2017). For example, hydrodynamic models demonstrated that in rivers affected by hydropeaking more heterogeneous habitats with alternating gravel bars created a more unstable flow environment, when compared to reaches with point bars (Hauer et al., 2014). Specific guidelines and habitat mitigation measures have been proposed for salmonid species not only according to refuge preferences tested in indoor flumes (Ribi et al., 2014), but also after studying hypothetical scenarios existing in natural rivers using hydrodynamic models (Almeida et al., 2017). Grounded in this knowledge, the dimensions, spatial arrangement and number of deflectors proposed in this study should be assessed according to the peaking flow conditions together with model simulations. Furthermore, the resultant mitigation structures should assure velocity refuges during up-ramping and water connectivity during down-ramping. Even recognizing the limitations of the flume size, it was possible to adjust the opening angle and length of the deflectors according to the size of young adults of *L. bocagei*. The vicinity and the downstream edge of the deflectors, are prone to the formation of eddies from which fish can benefit (Liao, 2007; Santos et al., 2014), and with the potential to increase their

attraction to the deflectors. In practice, in a natural context, the distance from the river bank to the edge of the deflector (determined by the opening angle of the deflector) should be at least in the same order of magnitude as the fish body length (Santos et al., 2014), thus not requiring overly wide angles in relation to the river bank. However, as this species often occurs in schools, the opening angle and dimension of the deflectors should also consider the group size. In contrast to wider angled structures (Hauer et al., 2017) which promote clogging associated with accumulated driftwood (Ribi et al., 2014), the proposed opening angle would guide the flow, reducing the deposition of fine sediment. To avoid fish stranding during the critical down-ramping, it should be guaranteed that the area behind the deflectors would not allow the formation of potential stranding zones, or assure a minimum water depth of 0.5 m (Almeida et al., 2017; Ribi et al., 2014).

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3.2.9 References

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4

Defining mitigation measures to hydropeaking supported by fluid-body interactions

"To know the spirit of a place is to realize that you are a part of a part and that the whole is made of parts, each of which in a whole. You start with the part you are whole in."

Gary Snyder

4.1 Fish under pressure: examining behavioural responses of Iberian barbel under simulated hydropeaking with instream structures

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4.1.1 Abstract

Hydropeaking is the rapid change in the water flow downstream of a hydropower plant, driven by changes in daily electricity demand. These fluctuations may produce negative effects in freshwater fish. To minimize these impacts, previous studies have proposed habitat enhancement structures as potential mitigation measures for salmonids. However, the recommendation of these mitigation measures for cyprinids remains scarce and their effects unknown. In this study, the effects of potential habitat mitigation structures under simulated hydropeaking and base-flow conditions are examined for Iberian barbel (*Luciobarbus bocagei*) in an indoor flume. Solid triangular pyramids and v-shaped structures were evaluated as potential flow-refuging areas and compared with a configuration without structures. A novel, interdisciplinary approach is applied to investigate individual and group responses to rapidly changing flows, by assessing physiological (glucose and lactate), movement behaviour (structure use, sprints and drifts) and the pressure distribution using a fish-inspired artificial lateral line flow sensor. The major findings of this study are four-fold: 1) Under hydropeaking conditions, the v-shaped structures triggered a lactate response and stimulated individual structure use, whereas solid structures did not elicit physiological adjustments and favoured individual and group structure use. Overall, both solid structures and their absence stimulated sprints and drifts. 2) The hydrodynamic conditions created in hydropeaking did not always reflect increased physiological responses or swimming activity. 3) Each event-structure combination resulted in unique hydrodynamic conditions which were reflected in the different fish responses. 4) The most relevant flow variable measured was the pressure asymmetry, which is caused by the vortex size and shedding frequency of the structures. Considering the non-uniform nature of hydropeaking events, and the observation that the fish responded differently to specific flow event-structure combinations, a diverse set of instream structures should be considered for habitat-based hydropeaking mitigation measures for Iberian barbel.

Keywords

Hydropeaking, Hydropower, Refuge, Iberian barbel, Artificial lateral line, Locomotion, Behaviour

4.1.2 Introduction

Hydropower provides an immediate and renewable source of electricity capable of responding to rapid daily fluctuations in electricity network demand. Accordingly, the construction of hydropower plants has continued worldwide (Nilsson et al., 2005; Zarfl et al., 2015). Due to the high fluctuations in daily electricity demand, the controlled discharge of water through the turbines creates hydropeaking, manifested as rapid flow fluctuations in the receiving water (Cushman, 1985). The rapid flow fluctuations result from the distinct phases of hydropeaking: base-flow discharge (no electricity production), increasing discharge or up-ramping (rapid increase in electricity production), continuous high peak discharge (peak energy demand), and decreasing discharge or down-ramping (shutdown of the turbines) (Tonolla et al., 2017). The range of flow alterations depends highly on the operation scheme of the hydropower plant. In regulated rivers having hydropower plants with inter-annual storage capacity, the peak-flows can exceed the base-flow by a factor of eight (Bejarano et al., 2017). The effects of these changes can be particularly noticeable in Iberian rivers affected by Mediterranean climate, where in summer there is low water availability in comparison with winter, and the environmental flows cannot exceed those of the natural flow regime (Gasith and Resh, 1999). For example, during summer periods the flow ratio can be eightfold, the daily peak frequency twofold, and peak duration one to two hours (Boavida et al., 2015). These fluctuations affect the ecological integrity of river ecosystems (Bunn and Arthington, 2002; Tuhtan, 2011) by changing the downstream morphological and hydrological processes (Bejarano et al., 2018; Greimel et al., 2018; Schmutz et al., 2015; Shen and Diplas 2010; Zimmerman et al., 2010; Zolezzi et al., 2011). Specifically addressing freshwater fish, those changes will affect diel activities (e.g. predator avoidance, foraging or finding refuge) and life-cycle events (e.g. reproductive migratory cues, survival or growth). These activities are intrinsically associated with movement behaviour shifts which differ from those occurring within natural flow conditions (for example, in magnitude and distance covered). Thus, fish are forced to adapt, and the energetic resources necessary to complete those events may be reduced (Flodmark et al., 2002). Due to their high mobility in the river system, fish reflect the longitudinal gradient of the river continuum. Thus, the biological impacts of hydropeaking have been mainly studied considering fish as the indicator organism (Bejarano et al., 2018). However, extreme macroinvertebrate drifts (Bruno et al., 2014) and physiological and physical constraints in riparian plants (Bejarano et al., 2018) have been documented.

Fish responses range from sub-organismal (e.g. neuroendocrine or metabolic adjustments) (Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012), to changes in key-life events (Burnett et al., 2014;

Casas-Mulet et al., 2015; Kelly et al., 2017; Korman et al., 2011). Sub-organismal responses follow a neuroendocrine pathway, initiated by the stimulation of the hypothalamic-pituitary-interrenal axis, which aim to restore the homeostatic state (Pankhurst, 2011). Negative effects occur when the organism is no longer capable of maintaining or recovering to that state, with repercussions on key life-cycle events, such as reproduction, growth, and survival (Barton et al., 2002; Pankhurst, 2011). To consider the broad range of biological responses, several experimental approaches have been adopted to study the effects of hydropeaking. Artificial channels (Arnekleiv et al., 2004; Auer et al., 2017; Costa et al., 2018a,b; Flodmark et al., 2002) and *in situ* rivers experiments (Krimmer et al., 2011; Taylor et al., 2013, 2012; Taylor and Cooke, 2012) have been conducted to investigate hydropeaking flow pulses and study the resulting fish behaviour. However, the direction and strength of specific flow-behaviour causal pathways may be identified by constraining external environmental factors under laboratory settings. Both in artificial channels and in natural conditions, transient cortisol elevations (Flodmark et al., 2002; Taylor et al., 2012), low lactate levels (Krimmer et al., 2011), and insignificant glucose changes (Flodmark et al., 2002; Krimmer et al., 2011) have been reported. In artificial channels, the cortisol changes were attributed to the induced flow fluctuations (Flodmark et al., 2002) whereas in natural conditions they were attributed to routine physiological processes, rather than to hydropeaking (Taylor et al., 2012). In most cases it has been difficult to establish a causal relation between flow variability and potential stress responses. Reported explanations for this difficulty include the time from stimulus perception to sample collection, the effects of other environmental and biological factors, and the experimental conditions (laboratory *vs.* field experiments) (Costa et al., 2017). These studies suggest that hydropeaking can trigger elevations in physiological responses. However, the direction and range of responses is difficult to determine. Nonetheless, it is expected that the physiological responses under hydropeaking conditions will be higher than those in base-flow conditions.

Changes in locomotor activity are detectable as immediate responses to rapid flow fluctuations. In rivers affected by hydropeaking, these changes can be manifested as local (Krimmer et al., 2011; Scruton et al., 2003; Taylor et al., 2014, 2013; Thompson et al., 2011) or large-scale spatial scale-movements (Boavida et al., 2017; Burnett et al., 2014; Capra et al., 2017; Harvey-Lavoie et al., 2016; De Vocht and Baras, 2003). However, no changes in the movement behaviour (Jones and Petreman, 2015), as well as changes which were difficult to interpret (Scruton et al., 2005) have also been reported. The diversity of movement behaviour responses has been largely attributed to physical and biological factors that were not possible to control, such as the presence of velocity refuges, and inter and intra-individual variability (Krimmer et al., 2011; Taylor et al., 2013). In addition to these, other physical (e.g. water quality, substrate, sediment dynamics, and hyporheic flow) and biological factors (e.g. species interactions) also affect fish movement behaviour and are difficult to reproduce in experimental flumes (Harby and Noack

2013). It is also important to note that laboratory studies cannot reproduce the full range of physical and biological factors driving fish behaviour as they exist in a river system. Nevertheless, indoor experiments have demonstrated the ability to study small-scale behavioural changes because of the possibility to control external confounding factors (Young et al., 2011). For example, simulated single peak events, consisting of an up-ramping stage followed by a continuous high peak, caused movement behaviour changes ranging from downstream displacement (Auer et al., 2017) to significant use of refuges (Ribi et al., 2014). This was in contrast to experiments with up- and down-ramping events, which did not find significant movement behaviour alterations (Cocherell et al., 2010; Flodmark et al., 2006). Under controlled conditions it is possible to visualize the behavioural diversity found in nature, however it is challenging to find ecological consequences for such behaviours (Mittelbach et al., 2014).

Only a handful of studies have investigated habitat enhancement structures based on fish behavioural changes to simulated rapidly changing flows Lateral refuges were effectively used by Iberian barbels for flow-refuging in simulated hydropeaking conditions (Costa et al., 2018a). T-shaped structures (Vehanen et al., 2000) and lateral refuges (Ribi et al., 2014) were suggested as potential flow-refuging areas for brown trout subjected to simulated hydropeaking. Although Ribi et al. (2014) demonstrated that brown trout used the lateral refuges for flow-refuging, Vehanen et al. (2000) reported high site fidelity of juveniles of this species, rather than using the refuge areas to compensate for the fluctuating flows. Similar behaviour was also described by (Kemp et al., 2003) for Atlantic salmon parr subjected to increased discharges in experimental flumes. The authors suggested that the short duration of the simulated flow events and the low habitat heterogeneity offered by artificial flumes effectively reduced the possibility of salmonid species to exhibit their full potential of movement behaviour patterns (Kemp et al., 2003; Vehanen et al., 2000). Previous studies on the effects of hydropeaking are focused mainly on salmonid species, whereas behavioural changes of cyprinids have been scarcely studied.

A major challenge of all studies addressing rapid flow fluctuations and their impact on fish behaviour is the difficulty to attribute a behavioural response to a specific flow fluctuation. This mechanistic link is also difficult to find because the current methods to measure the flow field are based on point values or spatial distributions of flow variables which do not take into account the inherent fluid-body interaction between a fish and the surrounding flow field. Fish experience the surrounding flow via their octavolateralis afferent system, consisting of the lateral line and inner ear sense organs (Popper, 1996). The lateral line consists of a linear array of mechanoreceptors located along the body, allowing fish to sense the spatial gradients of the flow field (Dijkgraaf, 1963). For the majority of freshwater fish species, sensory cues correspond to near-body fluctuations ranging from 1 - 150 Hz (Bleckmann, 2008, 1994). This range covers natural hydrodynamic variability and enables fish to perceive the different

spatiotemporal scales of flow, its alterations, and its interaction to the hydrodynamic environment. In this work, we attempt to characterize the hydrodynamic conditions resulting from a continuous peak flow and the presence of structures, by using the principles of the mechanosensory system of fish, through an artificial lateral line probe (LLP). To date, the assessment of fish behaviour according to the changes in the hydraulic environment has relied solely on conventional point flow measurements (Branco et al., 2013a; Goettel et al., 2015; Santos et al., 2014). Such measurements only consider a single point in space, and rely on the velocity and corresponding turbulence metrics measured using an acoustic Doppler velocimeter or propeller. Point-based metrics neglect the fluid-body interactions which fish actually use to sense the flow, and the frequency range of velocity measurements most commonly spans 1-50 Hz, which at best covers the lower third of the sensory range of biological lateral lines (van Netten and McHenry, 2013). Artificial lateral lines probes (LLPs) have emerged as a potential solution to fill this measurement gap. These devices consist of streamlined bodies with electromechanical sensor arrays. In this work, we investigate the potential utility of pressure sensor based LLPs. The LLP measures the rapid flow changes (up to 200 Hz) around its body using six differential pressure sensors. The motivation of using a LLP is that it more closely mimics the spatial and temporal sensing capacity of fish in contrast with low-frequency, point measurement device (Tuhtan et al., 2018, 2017). In this study, the flow metrics derived from the LLP were based on previous work comparing the sensor data to fish behaviour in a vertical slot fishway, where the most significant variables were the mean pressure, the mean front fluctuations, and the mean pressure asymmetry (Fuentes-Pérez et al., 2018).

The aim of this study is to assess the physiological and behavioural responses of Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864) (hereafter *L. bocagei*) associated with simulated hydropeaking in the presence or absence of instream structures in an indoor flume. Three hypotheses were investigated: 1) Rapid flow changes trigger physiological responses in *L. bocagei.* 2) Movement behaviour is affected by, and differs between simulated rapid flow changes and constant flow events with instream structures. 3) Critical thresholds of local hydrodynamic variables exist for *L. bocagei*, and are associated with distinct behavioural responses.

4.1.3 Methods

Ethics statement

All procedures involving animal manipulation, from capture in their natural environment to holding in the laboratory, were carried out in strict accordance with European standards (CEN, 2003) and Portuguese protocols (INAG, 2008) that assure animal welfare. Fish capture, handling,

transportation and holding permits were issued by the Institute for Conservation of Nature and Forests (ICNF) (permit numbers 290/2016/CAPT and 291/2016/CAPT). These permits also authorized the Laboratory of Hydraulics and Environment to hold *L. bocagei* in captivity for no longer than 10 days in accordance with the methodology described for this study. The experiments were conducted according to strict recommendations for the ''protection of animal use for experimental and scientific work'' (nº 5, article nº 31, Decree-Law 113/2013, 7th of August, transposing the European Directive n.º 2010/63/UE). In accordance with this Decree-Law, specific permits were issued by the nominated competent authority, the Direção-Geral de Alimentação e Veterinária (DGAV) (point a), article nº 3), which authorized IB and MJC to perform animal manipulation and procedures that involved animal health and welfare, and the conceptualization of projects that involved animal manipulation (nº 2, article nº 31). After revising the above-mentioned documents, the Ethics Committee of Instituto Superior Técnico (EC-IST) has given approval to carry out the experiments in the Laboratory of Hydraulics (Ref. nº6/2018 (CE-IST). Thus, all the necessary procedures to complete this study were authorized and performed with minimum handling stress. No fish were sacrificed for the purpose of this study.

Fish sampling and handling

The Iberian barbel (*Luciobarbus bocagei* Steindachner, 1864) was selected to study the behavioural and physiological responses to simulated hydropeaking. Little is known about the physiological responses to flow variability by *L. bocagei*. However, recent studies have reported increased glucose levels when *L. bocagei* was subjected to simulated hydropeaking (Costa et al., 2018a, b), as well as differences in lactate levels associated with successful fishway passage between seasons (Romão et al., 2018). This endemic cyprinid of the Iberian Peninsula is widely distributed in the river basins of northern and central Portugal (Oliveira et al., 2002) (IUCN status: least concern). *L. bocagei* is a bottom-oriented, potamodromous cyprinid (Branco et al., 2013b; Chan, 2010) feeding continuously during the day on benthic invertebrates and plant material, and adapting its diet according to the available food resources (Collares-Pereira et al., 1996; Magalhães, 1992). Fingerlings and juveniles are predominantly rheophilic (Martínez-Capel and García de Jalón, 1999; Santos et al., 2017), as are the spawning adults, which prefer fast-moving currents during upstream migrations (Rodriguez-Ruiz et al., 1992). Outside the reproductive season, the adults of *L. bocagei* tend to be limnophilic. In this study, young adults of *L. bocagei* were used because they are adapted to fast-moving flow conditions. Their habitat preferences change with ontogeny; juveniles prefer sand-gravel substrates and shallower areas, whereas adults prefer deeper areas with available refuges (Martínez-Capel and García de Jalón, 1999).

Fish were obtained at the Sorraia River (39.011376 º N, -8.357126 º W), a tributary of the Tagus River (central Portugal). The selected site is not affected by hydropeaking, which makes it a suitable source of unconditioned fish to study the impacts of hydropeaking. Fish were captured once a week in non-consecutive weeks between the 14th of October and the 18th of November of 2016 using a low-voltage (400 V) electrofishing gear (Hans Grassl IG-200), according to European norms (CEN, 2003) and national guidelines (INAG, 2008). No more than 80 fish were captured per each sampling occasion. In total, 150 fish were captured (mean total length ± SD = 14.7 ± 1.6 cm). After electrofishing, fish were transported (50 minutes) in a constantly aerated fish transport tank (Linn Thermoport 190 l) to the laboratory.

In the laboratory, the fish were distributed between two 900 l continuously aerated and biologically filtered holding tanks, and acclimated to ambient temperature and natural photoperiod for a 48-72 h period. Each holding tank was covered with a sunshade mesh, and clay roof tiles were placed in the bottom of the tanks to provide refuge areas. Water quality parameters (mean ± SD) were measured and adjusted in a daily basis using a multi parameter probe (YSI 556 MPS) for temperature (21.4 ± 1.7 ºC), pH (6.79 ± 0.28), dissolved oxygen (87.3 ± 5.8 %) and conductivity (304.2 ± 38.6 µS.cm^{-1}), and on a weekly basis using photometry (WTW- Spectroflex 6600) for nitrites (0.021 ± 0.004 mg.l^{-1}) and ammonia (0.001 ± 0.001 mg.l^{-1}). Partial water changes (15%) were performed every other day. The flume water temperature (24.0 ± 1.7 ºC) and dissolved oxygen (87.6 ± 6.4 %) were monitored twice a day. The water quality parameters were in accordance with the national legislation for water quality standards to protect and improve the aquatic environment according to water use (Ministry of the Environment, 1998). Fish were fed with a commercial diet for benthic species every day at night to avoid additional stress caused by food deprivation. Feeding took place only after the acclimation period.

Experimental facilities

The experiments were conducted between the 17th of October and the 23rd of November 2016 in an indoor flume located at the Laboratory of Hydraulics, at the University of Lisbon, Portugal (Figure 4.1). The flume has a rectangular cross-section, constructed on a steel frame with glass panels on both sides. The flume length was shortened to 6.5 m using two perforated metallic panels, and the flume width was 0.7 m (Figure 4.1). The discharge and the water level were controlled by a sluice gate upstream and by a flap gate downstream. The maximum discharge was 60 l.s^{-1}. Two plywood instream structures were designed and tested separately as potential refuges (solid triangular pyramids (R1) and v-shaped structures (R2), and a configuration without instream structures (R0) was also investigated (Figure 4.1).

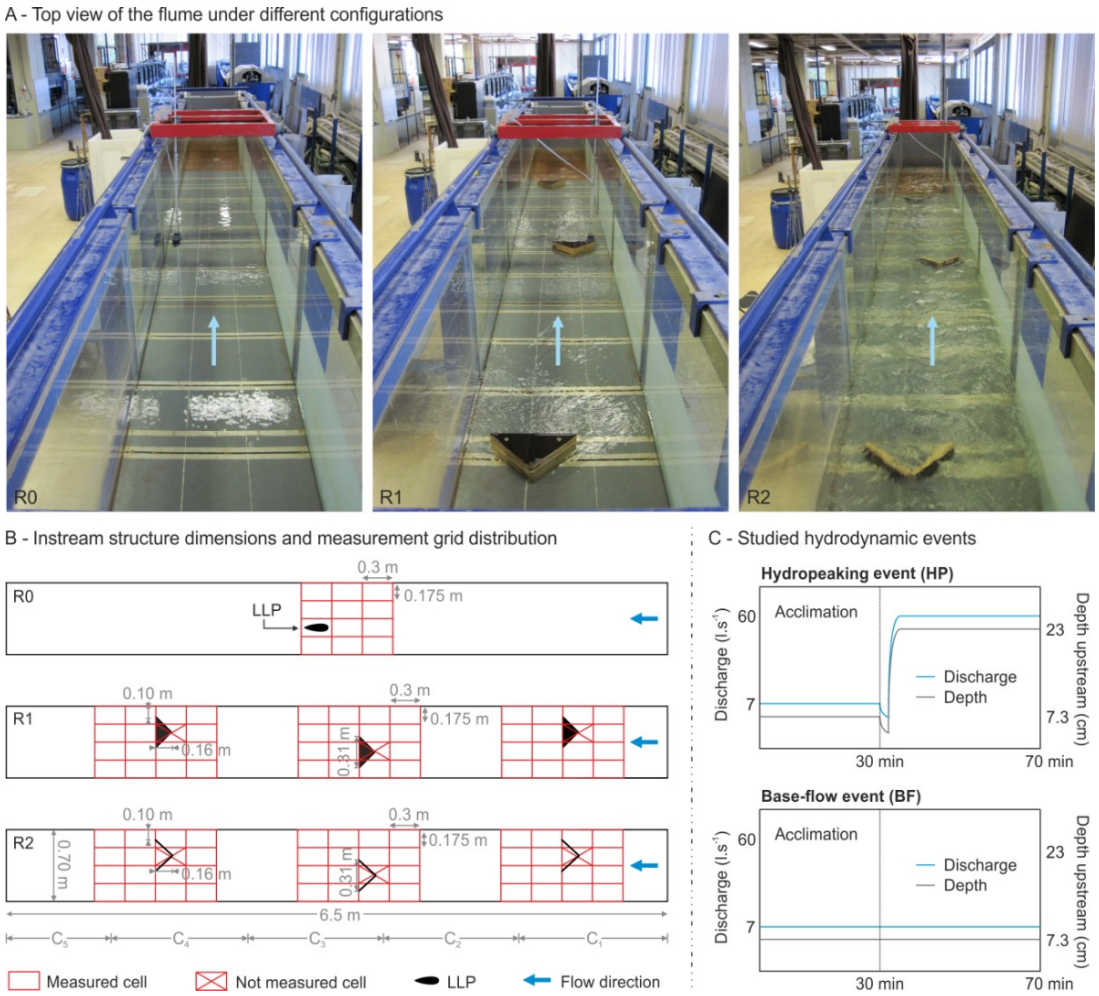

Figure 4.1 Summary of the experimental setup. A) Top view of the flume and test configurations (R0, R1 and R2). B) Plan of the setup with dimensions, grid of hydrodynamic measurements, and observation zones (C1 to C5). C) Depth and discharge of the studied hydrodynamic events.

Preferable substrate sizes should be at least in the same range as the fish length (Monk, 2012; Stevenson and Baker, 2009). Thus, the refuge shape and dimensions of this study were chosen to have at least the same order of magnitude as the fish´s body length (~ 20 cm) (Santos et al., 2014). The triangular shape creates a low velocity refuge immediately downstream of the structure and induces local flow areas in the wake with reduced vorticity (Heimerl et al., 2008). Likewise, the total number of structures was based on the spatial density of refuges according to experimental results in fish passages (Santos et al., 2014). The instream structures were placed in an alternating pattern, assuring that they were permanently wetted during base-flow conditions. This spatial arrangement intended to mimic a semi-natural displacement of substrate in the main river channel that may be exploited by fish for flow-refuging.

Hydrodynamic events

The flow ratio was 8.6, defined as the peak discharge (60 $l.s^{-1}$) divided by the base-flow discharge (7 $l.s^{-1}$) (Sauterleute and Charmasson, 2014). This value is considered high for fish species according to the literature (Harby and Noack, 2013; Sauterleute and Charmasson, 2014). Two flow events were simulated; hydropeaking (HP) and base-flow events (BF) (Figure 4.1C). Before each event, *L. bocagei* acclimated in the flume for 30 minutes with a flow rate of 7 $l.s^{-1}$ (Figure 4.1C). During this period, the flume upstream gate was open with a 10^{o} angle. The downstream gate was fixed at a 76^{o} angle during all experiments. The HP event consisted of a single up-ramping event where *L. bocagei* were subjected to the peak discharge for 40 minutes after the acclimation period. To create this event, the upstream gate was shut completely while filling the flume reservoir. The discharge was manually controlled until attaining 60 $l.s^{-1}$. Afterwards, the upstream gate was rapidly opened to 10^{o} to release the peak flow and reach the constant flow regime. The mean time (± SD) for this procedure (up-ramping) was 28.12 ± 1.86 seconds, corresponding to an up-ramping rate of 0.55 $cm.s^{-1}$ with a total increase of 15.7 cm in the water depth (rate of change) until reaching the constant flow regime. The BF event consisted of a continuous 7 $l.s^{-1}$ discharge stimulus for 40 minutes corresponding to the absence of hydropeaking conditions. The combination of instream structures (i.e. R0, R1, and R2) with flow events (i.e. HP and BF) resulted in five different sets of experiments, as follows: R0HP, R1HP, R1BF, R2HP and R2BF.

For each event, a school of five fish was tested and replicated five times. Each fish was tested only once. It should be noted that larger groups of *L. bocagei* occur in nature, particularly during the reproductive season. A school size of five fish was selected to optimize the observation of fish movement behaviour in the flume. This was done in order to reproduce representative group behaviour and to reduce the number of captured fish.

Fish responses

Physiological responses

To evaluate the potential physiological responses to hydropeaking between instream structure configurations (R0 *vs.* R1 and R2), the levels of blood glucose and lactate were quantified. These physiological parameters were chosen since glucose and lactate level increases are usually directly associated with primary responses to stress (Pankhurst, 2011). Additionally, changes in glucose and lactate levels have been widely used as secondary physiological indicators of stress to flow variability

(Costa et al., 2017). Glucose and lactate levels increases are usually directly associated with primary responses to stress (Pankhurst, 2011). Lactate increases have been associated with prolonged anaerobic swimming when the organism can no longer maintain aerobic sustained swimming, resulting in exhaustion (Wendelaar Bonga, 1997). Hence, both physiological responses may represent reliable surrogates of a stress response to flow variability. After each flow event, each fish was dip-netted from the flume and transferred to a recipient with permanently oxygenated water and immediately placed in a v-shaped plastic trough in a supine position. Blood samples (0.1-0.5 ml) were collected via caudal puncture using 23 G or 25 G pre-heparinized needles. It has been demonstrated that three minutes is not long enough to have a significant influence on primary stress responses (e.g. cortisol) (Sumpter, 1997). The glucose and lactate levels were immediately measured using the portable meters Accu-check Aviva (Roche) and Lactate Plus (Nova Biomedical UK) respectively. These lactate and glucose portable meters have been successfully tested and validated for fish research (Beecham et al., 2006; Stoot et al., 2014; Tanner et al., 2010).

Movement behaviour

To examine the effects of instream structure type and flow event on *L. bocagei*, the behaviour metrics were selected based on fish body motion directed towards the structure and to changes in motion corresponding to the occurrence of a peak event. Afterwards, the behaviour metrics were divided into two categories: 1) structure use, and 2) swimming activity in the flume (Figure 4.2). The frequency of each behaviour was defined as the number of occurrences, in absolute frequency, over the duration of the flow event (i.e. 40 minutes). Each metric was attributed to a single fish (I) or to a group of two to five fish (G).

Successful structure use was considered when a single fish (Iref, Figure 4.2A) or a group of fish (Gref, Figure 4.2B) were observed in the immediate downstream area of the structure (Figure 4.2A-B). Considering the position of each structure in the flume, there were two possibilities for *L. bocagei* to pass by them and move upstream: by using the shortest (0.10 m) or the longest (0.29 m) distances between the structures and the correspondent flume wall (Figure 4.2C-E). This positive rheotactic movement was registered when a single or a group of fish used either the shortest (Iwall, Figure 4.2E; Gwall, Figure 4.2F) or the longest (Imiddle; Figure 4.2C; Gmiddle, Figure 4.2D) distances respectively.

All swimming activity metrics were selected to represent movement responses which may be associated with the severity of a peak event. They were separated into two types; fish sprints, defined as a swimming activity lasting a few seconds and characterized by several tail beats (Isprint, Figure 4.2I, and

Gsprint, Figure 4.2J), and fish drifts, defined as downstream fish displacements driven by passive advection of the body in the flow direction (Idrift, Figure 4.2G, and Gdrift, Figure 4.2H).

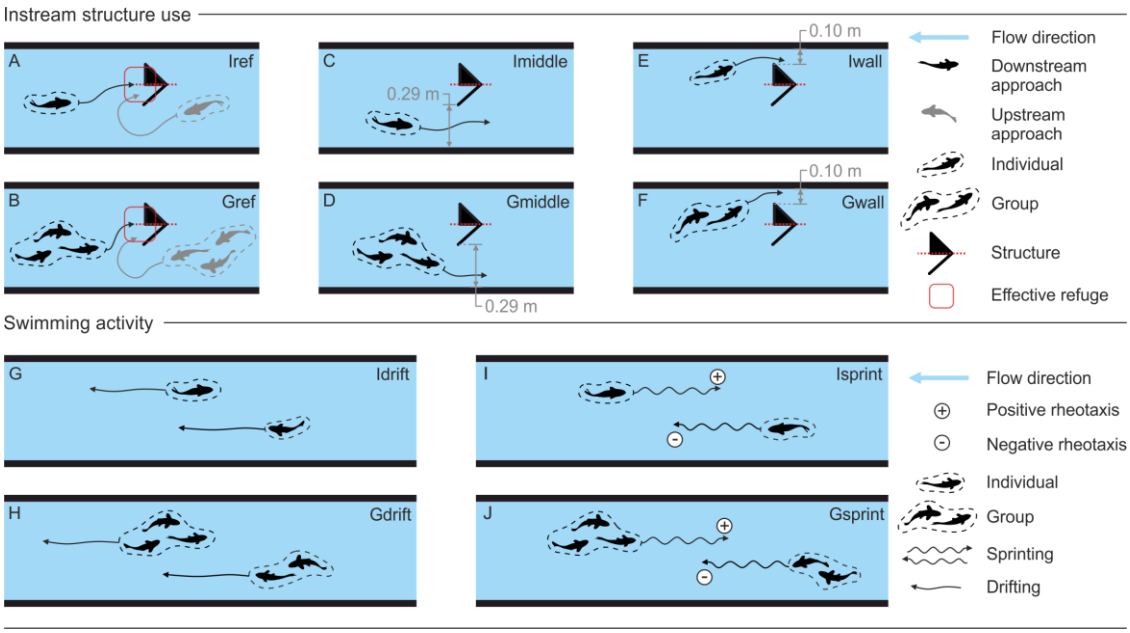

Figure 4.2 Classification of the behaviour metrics. (A and B) - Individual (Iref) and group (Gref) structure use; (C and D) – Individual (Imiddle) and group (Gmiddle) passage by the structures using longest distance between the structure and the flume wall; (E and F) – Individual (Iwall) and group (Gwall) passage by the structures using the shortest distance between the wall and the structure. (G and H) Individual (Idrift) and group (Gdrift) drifts. (I and J) Individual (Isprint) and group (Gsprint) sprints. Plus (+) and minus (-) signs represent the swimming direction of L. bocagei, as positive and negative rheotaxis respectively. These metrics were used for all configurations.

Activity metrics were counted considering the total flume usable area (observation zones C1 to C5, Figure 4.1B). A behaviour occurrence was only assigned to a specific observation zone only if it began at that location. Each individual or group were considered a sampling unit and any individual behaviour was registered if performed by one fish independently of the group.

Hydrodynamic characterization using an artificial lateral line

An artificial lateral line probe (LLP) was used to measure the local flow field properties during the different flow events and quantify the effects of instream structures. LLPs are streamlined bodies with a pressure sensitive sensor array. The devices allow for a characterization of the fluid-body interactions, and are capable of measuring at the same rates as the natural sensing frequencies of fish, from 10s to 100s Hz (Fuentes-Pérez et al., 2015). The LLP used in this study consisted of a 0.22 m length NACA025 body which measures pressure gradients over the body with six differential pressure sensors (±2000 Pa MPXV7002). All measurements with the LLP were conducted using a 200 Hz sampling rate. In

addition, the water depth was measured by the probe using an absolute pressure sensor (0 to 10000 Pa - MPX5010GP) (Figure 4.3).

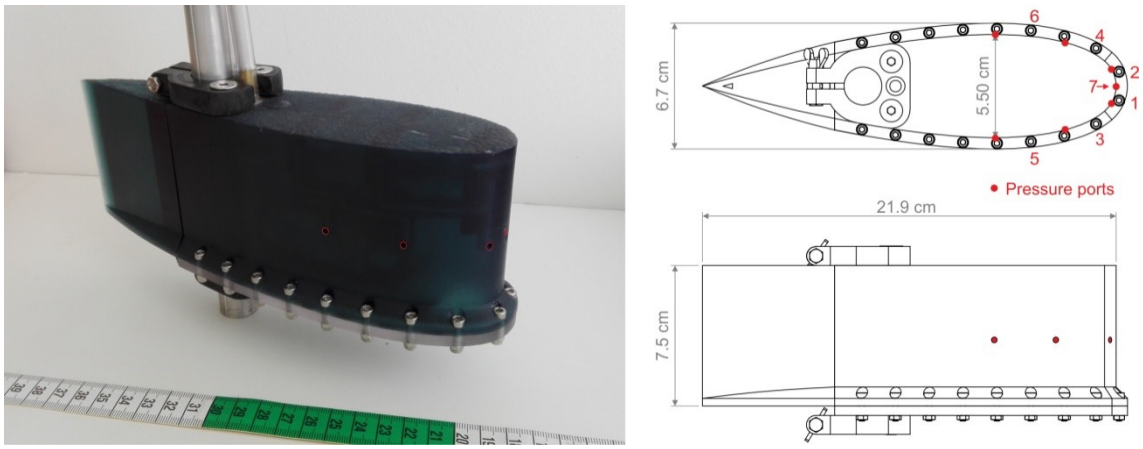

Figure 4.3 Lateral line probe (LLP). NACA025 body shape, showing the locations of the differential pressure sensors (1-6), and the absolute pressure sensor (7).

The selected variables were: mean front pressure, mean front fluctuations and mean pressure asymmetry (Table 4.1). The mean front pressure ($\overline{p}_{12}$) is quadratically related with the velocity of the flow, following the conservation of energy (Salumäe and Kruusmaa, 2013). This variable is also directly correlated with the free stream flow velocity experienced by the fish body. Higher mean front pressure magnitudes also imply an increase in the hydrodynamic drag, thus upstream movements may be reduced and increase the fish energy consumption during station holding (Tudorache et al., 2008). The mean front pressure fluctuations ($\overline{p}'_{12}$) represent the magnitude of the rapid changes of pressure gradients on the body of the LLP, and therefore, they are correlated with the turbulence experienced by the probe (Chen et al., 2017). They represent the changes in pressure magnitudes over time at a given location. In general, lower pressure magnitudes correspond to regions of uniform and stable flow conditions. Fish, and in particular *L. bocagei*, have shown preferences for low turbulence areas (Fuentes-Pérez et al., 2018; Silva et al., 2011). The mean pressure asymmetry ($\Delta\overline{p}'_{1-6}$) offers an instant comparison of the pressure gradient differences in each of the sides of the LLP. This allows the identification of cyclic flow patterns (e.g. repeating patterns of vortices) and their magnitude (Tuhtan et al., 2017). It is also worth noting that vortices of a scale in the same order of magnitude as a fish's body length may also be utilized to reduce swimming cost and can even be leveraged to enhance upstream movements (Liao, 2006).

Table 4.1 Pressure-based variables form LLP measurements used in this study. Definition of mean pressure and mean pressure fluctuations are included to clarify the mathematical definition of the variables used for this study. n: number of data points in a measurement; i: index of the sensor.

Variables	Equation
Mean pressure	$\overline{p}_i = \frac{\sum_{j=1}^{n} p_{i,j}}{n}$
Mean pressure fluctuations	$\overline{p}'_i = \frac{\sum_{j=1}^{n} \left\| p_{i,j} - \overline{p}_i \right\|}{n}$
Mean front pressure ($\overline{p}_{12}$)	$\overline{p}_{12} = \frac{\sum_{j=1}^{n} \frac{p_{1,j} + p_{2,j}}{2}}{n}$
Mean front pressure fluctuations ($\overline{p}'_{12}$)	$\overline{p}'_{12} = \frac{\overline{p}'_1 + \overline{p}'_2}{2}$
Mean pressure asymmetry ($\Delta\overline{p}_{1-6}$)	$\Delta\overline{p}_{1-6} = \frac{\sum_{k=1}^{m} \frac{\sum_{j=1}^{n} \left(p_{2k-1,j} - p_{2k,j} \right)^2}{n}}{3}$

The selected measurement grid (Figure 4.1) covered the regions most affected by the rapid flow changes as well as the peak discharge for R1 and R2. The grid was broken into volume elements whose lateral and streamwise dimensions closely match the body length of young adults of *L. bocagei* as well as the LLP.

Data analysis

A Kruskal-Wallis analysis was performed to verify whether the hydrodynamic event and structure type/presence triggered physiological responses in *L. bocagei*. The analysis determined if there were statistical differences in the levels of blood glucose and lactate among replicates of each event. As there was not any statistical evidence that supported that hypothesis, the lactate and glucose levels of each *L. bocagei* were considered as true replicates. Afterwards, to verify if there were any significant differences in the levels of blood glucose and lactate of *L. bocagei* among events, a Kruskal-Wallis test was applied with a Nemenyi post-hoc test for pairwise contrasts (Pohlert, 2014). Post-hoc tests for pairwise contrasts between events were made using the R-package PMCMR (Pohlert, 2014).

To provide a visual representation of the main trends for instream structure use (i.e. Iref, Gref, Imiddle, Gmiddle, Iwall, Gwall) and swimming activity (Isprint, Gsprint, Idrift, Gdrift) of *L. bocagei* according to the hydrodynamic events, a correspondence analysis (CA) was conducted. This method preserves the $\chi 2$

distance among objects, does not require data normalization, and it is suitable for frequency-like data (Borcard et al., 2011). CA is adequate for this study considering the nature of the behaviour response metrics (absolute frequency) and its reduced number (i.e., six for instream structure use, and four for swimming activity) (Borcard et al., 2011). This analysis was conducted using the R-package vegan (Oksanen et al., 2017). To test if the event was affecting (1) the frequency of instream structure use and (2) the swimming activity of *L. bocagei* the two-way distance-based multivariate analysis of variance based on Euclidean distance test was applied (using Euclidean distance and 999 permutations) (Oksanen, 2015). This method does not require the assumptions of parametric tests (Anderson, 2001), is suitable for small sample sizes (Walters and Coen, 2006), as well as continuous and factor predictors (Oksanen, 2015). The analysis was conducted using the R-package vegan (Oksanen et al., 2017). If an effect was detected, a detailed analysis per metric of refuge use and flume swimming activity was conducted by Kruskal-Wallis analysis with a post-hoc Nemenyi test for pairwise contrasts between events (Pohlert, 2014). The statistical analysis was performed using R version 3.3.2 (R Core Team, 2017). This analysis was conducted using the R-package PMCMR (Pohlert, 2014). All statistical analyses were performed using R version 3.3.2 (R Core Team, 2017).

The variables measured with the LLP were plotted onto the grid to illustrate and compare the differences among the studied events. A summary of the measured minimum values of the pressure variables in the area affected by the structures and the mean ± *SD* values in the flume area were considered. This was performed to simplify the discussion of the LLP results, compare relationships to the fish movement responses, and define local flow preferences for this fish species. The area affected by the structure consisted of 3 grid rectangles measured downstream and near the structure (3 rectangles behind each structure, n = 9), and the flume area consisted of the remaining grid rectangles (n = 45 - 9 = 36). All data analysis, plots, and calculations concerning LLP were performed using MATLAB release R2017a.

4.1.4 Results

Physiological responses

The glucose levels differed significantly among events ($\chi 2$ (4) = 10.870, p = 0.028) and were higher in *L. bocagei* subjected to R1BF in when compared to R0HP ($\chi 2$ = 4.331, p = 0.019) (Figure 4.4). The lactate levels differed significantly among events (χ^2 (4) = 17.141, p = 0.002) and were higher in R2HP compared to R0HP (χ^2 = 5.117, p = 0.003) and R1HP (χ^2 = 4.161, p = 0.027) (Figure 4.4B).

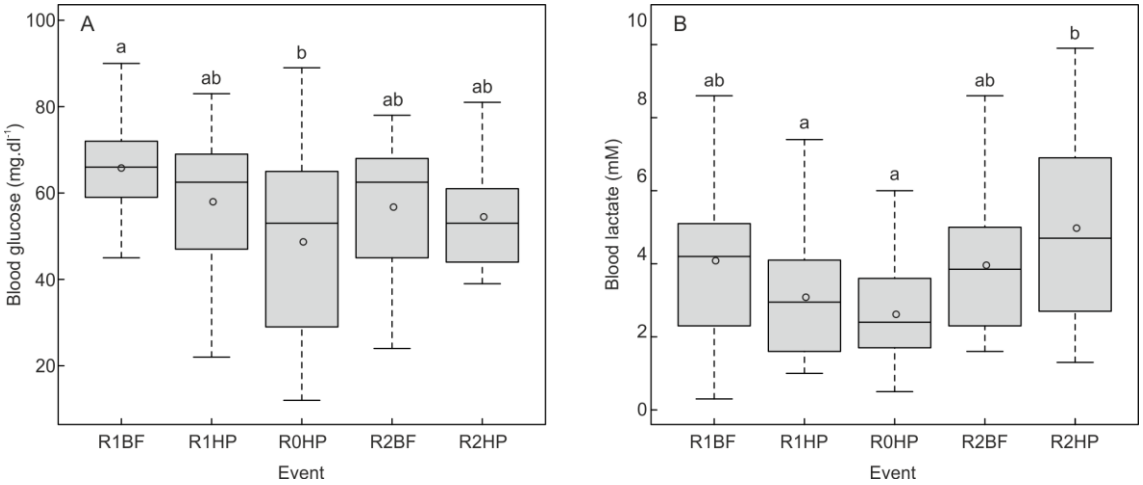

Figure 4.4 Boxplots with the variation of (A) blood glucose (mg.dl^{-1}) and (B) lactate (mM) levels for *L. bocagei*. R1BF and R2BF - Base flow event in the presence of solid triangular pyramids and v-shaped structures respectively; R0HP, R1HP and R2HP – Hydropeaking event in the absence of structures, presence of solid triangular pyramids and presence of v-shaped structures respectively. The letters correspond to the post-hoc test results. The circles correspond to the mean value of the physiological response for each treatment.

Movement behaviour

The CA orthogonal axes represent the main trends observed for the frequency of instream structure use and swimming activity in the flume according to the hydrodynamic events (Figure 4.5).

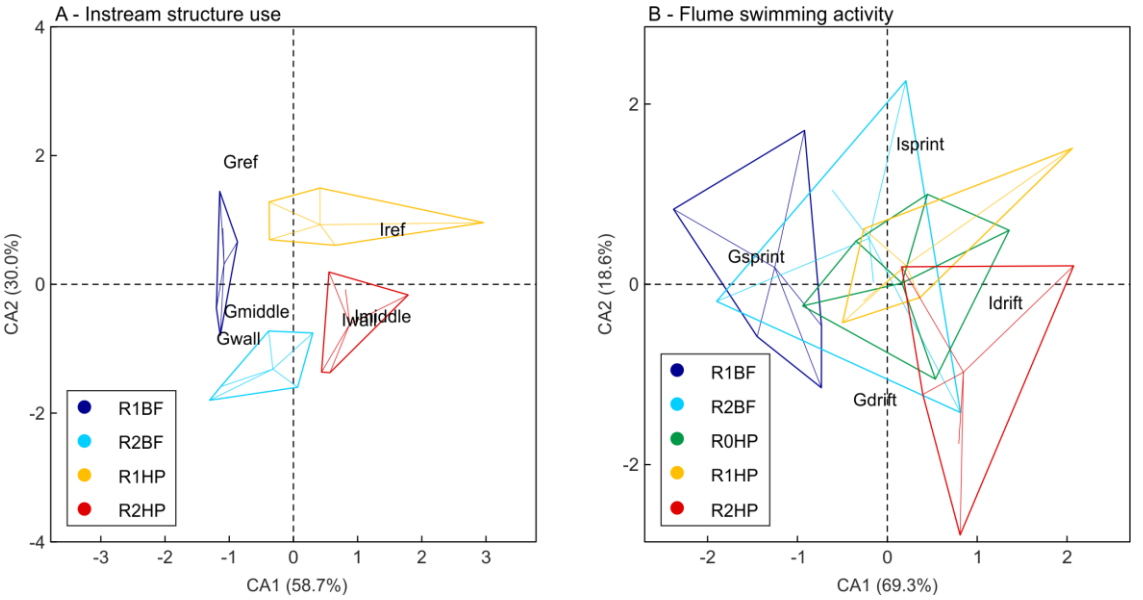

Figure 4.5 Correspondence Analysis (CA) biplots for the instream structure use (A) and swimming activity in the flume (B). The biplots illustrate the spatial distribution of behaviour according to each flow event. The axes labels indicate the proportion (%) that explains the spatial distribution of the events. R1BF and R2BF - Base flow event in the presence of solid triangular pyramids and v-shaped structures respectively; R0HP, R1HP and R2HP – Hydropeaking event in the absence of structures, presence of solid triangular pyramids and presence of v-shaped structures respectively.

The first two axis for the frequency of structure use explain 88.7 % of the variation among events (Figure 4.5A). BF opposes HP on the first CA axis, whereas R1 opposes R2 on the second CA axis (Figure 4.5A). A clear trend can be observed considering group structure use in BF in opposition to individual structure use in HP (Figure 4.5A). The spatial organization and the structure use scores, indicated that *L. bocagei* individuals more frequently make use of R1, particularly in R1HP (Figure 4.5A). The path chosen to cross the structures was more evident in the R2HP, particularly for individuals as well (Figure 4.5A). The first two axes for the swimming activity account for 87.9 % of the variation among events (Figure 4.5B). R1BF opposes to R2HP in the first CA axis for group *vs.* individual behaviour respectively (Figure 4.5B). Gsprint was the most frequent behaviour in R1BF, whereas Idrift was the most frequent behaviour in R2HP (Figure 4.5B). In the absence of instream structures, i.e. in R0HP, there was a more evenly distributed swimming activity pattern.

The ordination results were supported by the multivariate analysis, where a significant effect of flow event on the structure use (F = 5.156, p = 0.001) and swimming activity (F = 7.839, p = 0.001) of *L. bocagei*. There were significant differences in the mean frequency of individual (Iref; χ^2 (3) = 11.271, p = 0.010) and group structure use (Gref; χ^2 (3) = 12.233, p = 0.007) among flow events. Rank comparisons showed that Iref was higher for *L. bocagei* that were subjected to R1HP and R2HP in comparison with R2BF (p = 0.038 and p = 0.033 respectively) (Figure 4.6A).

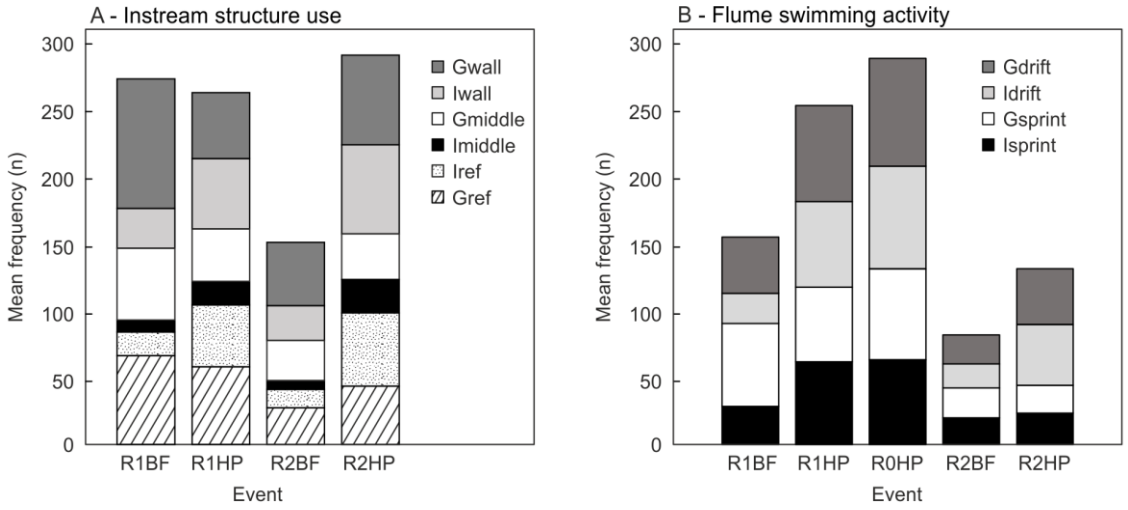

Figure 4.6 Mean frequency (n) of each behaviour metric for each event. A) Structure use (Iref and Gref), path chosen to cross the structures (Imiddle, Gmiddle, Iwall, Gwall). B) Flume swimming activity (Isprint, Gsprint, Idrift and Gdrift). R1BF and R2BF - Base flow event in the presence of solid triangular pyramids and v-shaped structures respectively; R0HP, R1HP and R2HP – Hydropeaking event in the absence of structures, presence of solid triangular pyramids and presence of v-shaped structures respectively.

Groups of *L. bocagei* used the downstream area of solid triangular pyramids more frequently in the base-flow (R1BF) and in the hydropeaking events (R1HP) in comparison with the base-flow in the presence of v-shaped structures (R2BF) (p = 0.008 and p = 0.044 respectively) (Figure 4.6A).

There were also significant differences in the path that fish selected to cross the structures for individual behaviour (Imiddle; χ^2 (3) = 9.398, p = 0.024; Iwall; χ^2 (3) = 8.504, p = 0.037), but not for group behaviour (Gmiddle: (χ^2 (3) = 5.597, p = 0.133; Gwall: (χ^2 (3) = 7.563, p = 0.056). The mean frequency of Imiddle was significantly higher for R2HP in comparison with R2BF (F= 3.817; p = 0.035) (Figure 4.6A). The mean frequency of flume swimming activity differed significantly among flow events and structure configurations for Isprint (χ^2 (4) = 16.525, p = 0.002), Gsprint (χ^2 (4) = 13.374, p = 0.010), Idrift (χ^2 (4) = 18.49, p = 0.001) and Grift (χ^2 (4) = 15.403, p = 0.004). The mean frequency of Isprint was significantly higher in R0HP (F = 4.375; p = 0.017) and R1HP (F = 4.010; p = 0.037) in comparison with R2BF (Figure 4.6B).The mean frequency of Idrift was significantly higher in R0HP (F = 4.466, p = 0.013) and R1HP (F = 3.859, p = 0.049) in comparison with R1BF, and in R0HP (F = 4.618, p = 0.010) and R1HP (F = 4.010, p = 0.037) in comparison with R2BF (Figure 4.6B). The mean frequency of Gdrift was significantly higher in R0HP (F = 4.739, p = 0.007) and R1HP (F = 4.618, p = 0.010) in comparison with R2BF (Figure 4.6B).

Three major trends were apparent after integrating the physiological and movement behaviour metrics: 1) Lower levels of glucose in R1BF were associated with an increased group structure use; 2) Higher lactate levels in R2HP where associated with increased individual attempts to cross the structures and move upstream; 3) The absence of significant differences in the levels of both glucose and lactate in R1HP and R0HP was associated with an increased structure use in R1HP, and in the drift frequency in R1HP and R0HP (Table 4.2).

Table 4.2 Summary of the physiological and behavioural responses of *L. bocagei* according for each event tested. Only the more pronounced fish responses with statistically significant differences are shown, demonstrating the unique fish response patterns according to each hydrodynamic event.

Tested event	**Fish responses**		
	Physiology	**Movement behaviour**	
		Structure use	**Swimming activity**
R1BF	↓Glucose[1]	Gref[3]	
R1HP		Gref[3]; Iref[3]	Isprint[3]; Idrift[4]; Gdrift[3]
R0HP		N/A	Isprint[3]; Idrift[4]; Gdrift[3]
R2BF			
R2HP	↑Lactate[2]	Iref[3]; Imiddle[3]	

[1] In comparison with R0HP; [2] in comparison with R1HP and R0HP; [3] in comparison with R2BF; [4] in comparison with R1BF and R2BF; N/A not applicable

Hydrodynamic characterization using an artificial lateral line

Figure 4.7Figure 4.8Figure 4.9 provide a representation of the pressure-based variables considered in this study: $\overline{p}_{12}$, $\overline{p}'_{12}$ and $\Delta\overline{p}_{1-6}$. The distribution of mean front pressure represents the total mean pressure difference experienced by the LLP in the front of its body ($\overline{p}_{12}$, Figure 4.7).

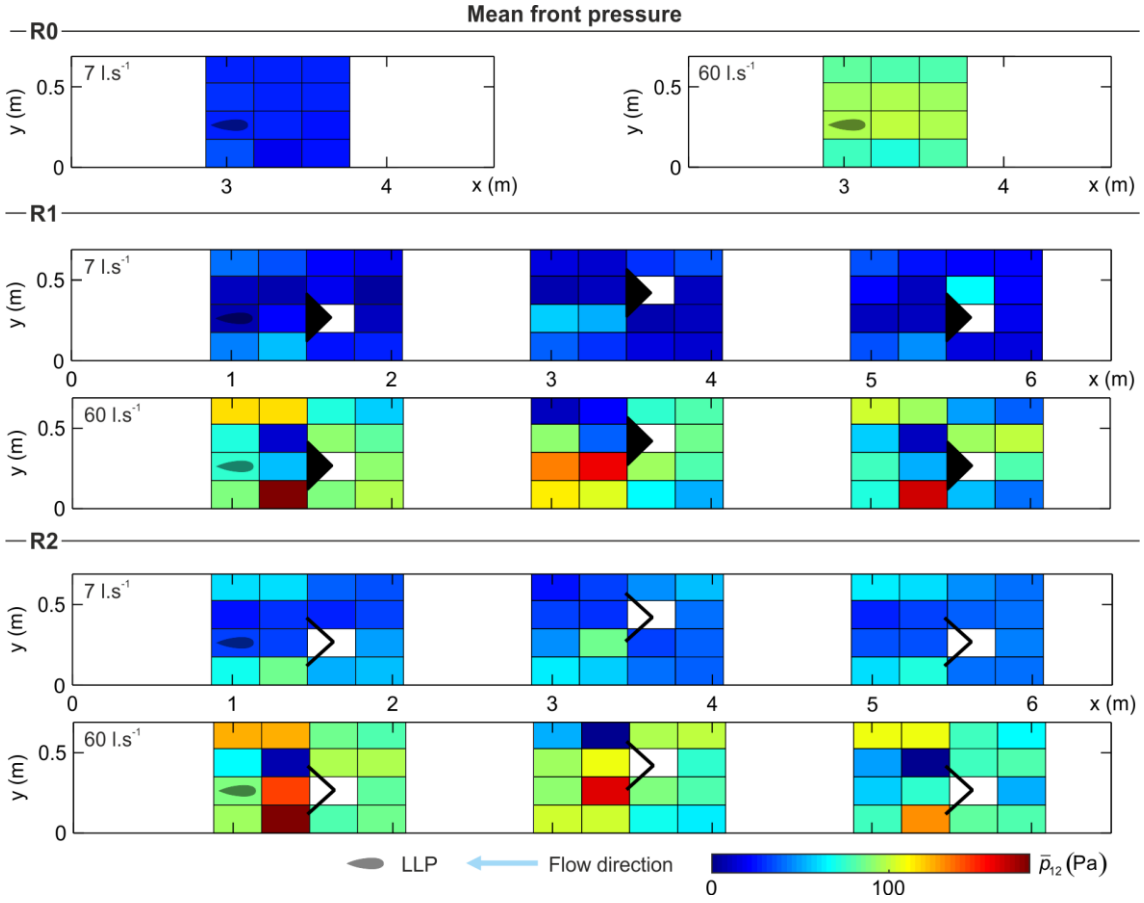

Figure 4.7 Distribution of mean front pressure ($\overline{p}_{12}$) for all configurations and both discharges scenarios considered (7 and 60 l.s^{-1}). The reader is referred to Appendix (Supplement 2) to look at the contour representation of this figure.

It was found that for all configurations (R0, R1 and R2) that an increase in discharge leads to higher values of $\overline{p}_{12}$. In the absence of structures, the discharge distributed uniformly over the cross-section and $\overline{p}_{12}$ increased solely as a function of discharge (R0, Figure 4.7). However, the presence of structures (R1 and R2) will generate a low $\overline{p}_{12}$ area immediately behind the structure (refuge effect), and areas of higher $\overline{p}_{12}$ occur due to a local flow acceleration adjacent to the structure (Figure 4.7, Table 4.3). The distribution of these flow-refuging areas downstream the structure is driven by the flow direction.

Table 4.3 Minimum (Pa) and mean ± SD (Pa) LLP derived pressure variables. The minimum pressure values (Pa) for mean front pressure ($\overline{p}_{12}$), mean front pressure fluctuations ($\overline{p}'_{12}$) and mean pressure asymmetry ($\Delta\overline{p}_{1-6}$) refer to the results observed in the three rectangles measured behind the structure, Mean ± SD (Pa) refer to the pressure values observed in the flume (excluding structure area) for events R1BF, R1HP, R0HP, R2BF and R2HP.

Tested event	LLP derived pressure variables					
	Structure area (minimum)			Flume (mean ± *SD*)		
	Front pressure (Velocity) (Pa)	Front fluctuations (Turbulence) (Pa)	Asymmetry (log Pa)	Front pressure (Velocity) (Pa)	Front fluctuations (Turbulence) (Pa)	Asymmetry (log Pa)
R1BF	5.70	1.72	8.39	23.33±14.17	9.04±6.19	9.27±0.19
R1HP	11.18	6.52	9.17	80.32±24.78	30.62±11.55	9.55±0.38
R0HP	-	-	-	87.21±9.92	18.13±1.92	2.08±0.01
R2BF	30.35	1.51	9.54	44.15±12.13	9.66±7.02	9.86±0.14
R2HP	-4.88	31.16	10.00	84.39±18.29	30.80±10.18	10.14±0.19

The mean front pressure fluctuations quantify the change of the pressure magnitude over time, with lower values indicating more stable zones ($\overline{p}'_{12}$, Figure 4.8).

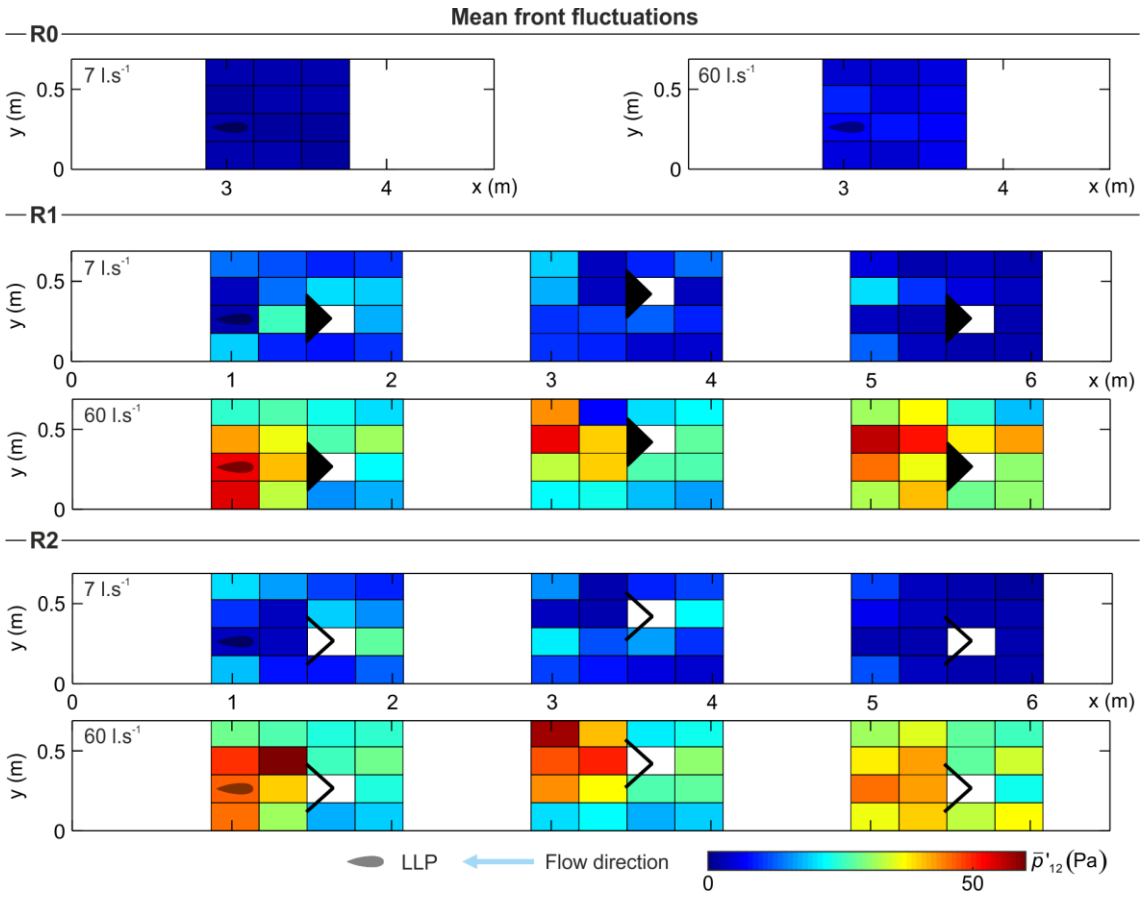

Figure 4.8 Distribution of mean front fluctuations ($\overline{p}'_{12}$) for all configurations and both discharges considered (7 and 60 l.s^{-1}). The reader is referred to Appendix (Supplement 3) to look at the contour representation of this figure.

In the absence of structures $\overline{p}'_{12}$ presented a uniform distribution driven only by the discharge and flume geometry (Figure 4.8). The presence of the structure generated a region of high-pressure fluctuations, $\overline{p}'_{12}$ (area of high turbulence) (R1 and R2, Figure 4.8, Table 4.3). The distance to this highly fluctuating area depended on the discharge. Lower fluctuations were found near, inside and immediately behind the structure (refuge effect), and between structure and walls due to the coherence created by converging streamlines.

The mean pressure asymmetry represents the instantaneous pressure differences between both sides of the probe, detecting and quantifying the vorticity experienced ($\Delta\overline{p}_{1-6}$, Figure 4.9). Similar to the other variables, its distribution shows that in the absence of structures, the asymmetry remains uniform and with low magnitude. This is primarily due to the absence of planar vortices shed by the structures (R0, Figure 4.9). In general, it was observed that $\Delta\overline{p}_{1-6}$ had a larger in R2 than R1, and increased slightly as a function of discharge.

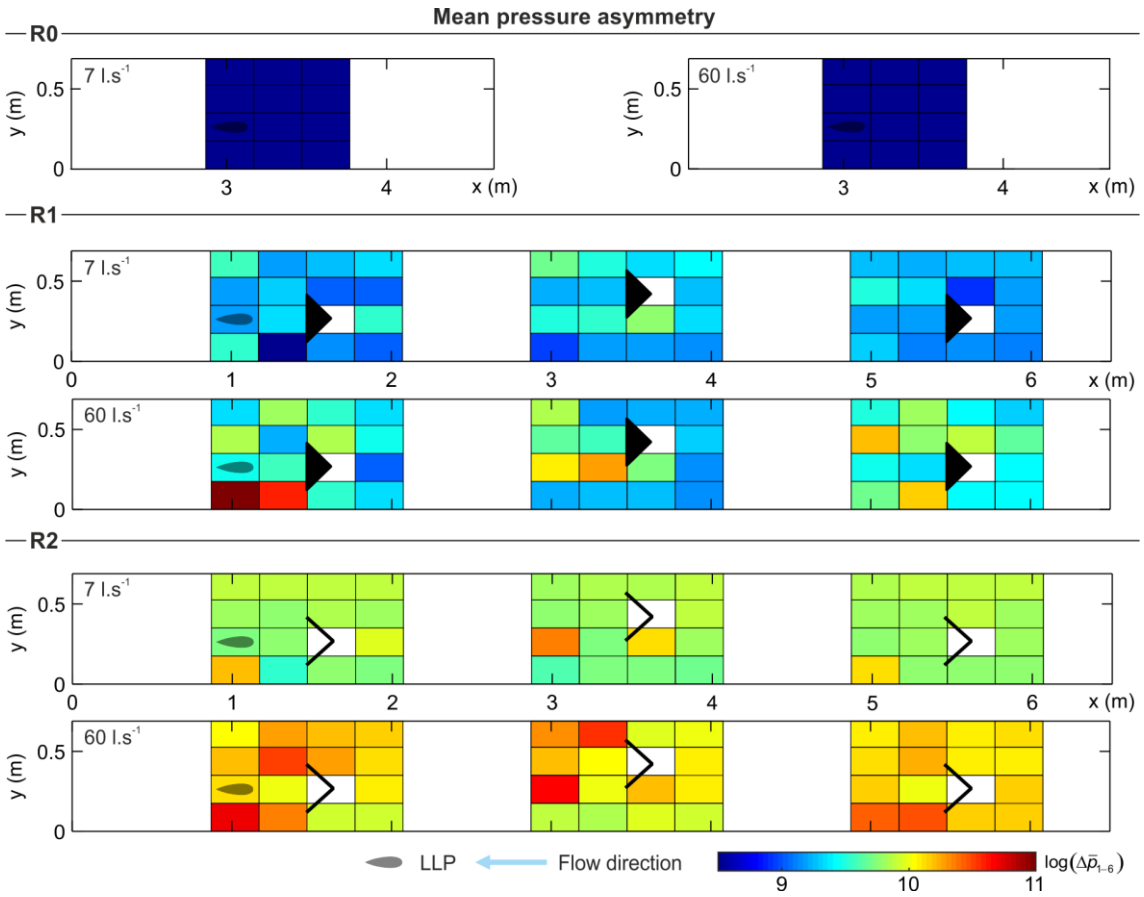

Figure 4.9 Distribution of mean pressure asymmetry ($\Delta\overline{p}_{1-6}$) for all configurations and both discharges scenarios considered (7 and 60 l.s^{-1}). The reader is referred to Appendix (Supplement 4) to look at the contour representation of this figure.

4.1.5 Discussion

The effects of simulated hydropeaking events including instream structures as flow refuges for *L. bocagei* were investigated. A novel, interdisciplinary approach combined physiological and behavioural responses with fluid-body interactions measured with a fish-inspired artificial lateral line. The combinations of hydrodynamic events and instream structures altered the spatio-temporal distribution and magnitude of velocity, turbulence fields and pressure fluctuations (Figure 4.7-4.9), and were found to correspond to distinct physiological and behavioural responses (Figure 4.4-4.6, Table 4.2).

The physiological and behavioural responses differed between hydropeaking and base-flow conditions as well as with instream structure configuration. An interesting finding was that although flow and behaviour were indeed linked, the extreme hydrodynamic changes did not always result in higher physiological levels or frequencies in movement behaviour (Figure 4.6, Table 4.2). Similarly, in rivers affected by hydropeaking, there were no significant physiological increments in salmonids possibly explained by flow-refuging, feeding and social interactions (Taylor et al., 2012). Although higher swimming costs are associated to high flows, fish can use these as migratory and spawning cues (Young et al., 2011), and use the low flows to optimize foraging behaviour (Larranaga et al., 2018). As an example, glucose and lactate levels were not systematically considering HP events. The results indicate that *L. bocagei* responses may be related to the combination of flow event severity and structure configuration, rather than solely to the severity of the flow event itself. Given this evidence, the lowered physiological responses and frequencies of movement behaviour observed in R2BF (particularly in comparison with R1BF) and the increased structure use and drift frequency (as a means to recover from the peak-flow conditions) in R1HP indicate that these event-configuration combinations can be favourable for *L. bocagei*.

The highest lactate levels were observed in R2HP. This can be explained by the higher frequency of passages observed in R2 (Figure 4.6A) together with the lower frequency of drifts (Figure 4.6B). An increase in observed swimming velocities corresponds to higher swimming costs (Beamish, 1978; Tang et al., 2000) indicating that the fish had difficulty maintaining their focal position while crossing the structures, which may explain the higher lactate levels. In addition, the lower frequency of drifts observed in R2HP when comparing with R0HP and R1HP (Figure 4.6B), and the higher lactate levels (Figure 4.4B) may indicate that the thresholds of pressure variables during this event, and in particular asymmetry (Figure 4.9) may have acted to hinder fish drifts. Although drifts are typically associated with the inability to hold position, the lactate elevations denote increasing swimming effort. These results suggest that the hydrodynamics shed by the v-shaped structures could have hindered the use of these

structures as flow-refuging areas. Furthermore, the observed drifting behaviour may provide a means to recover from the additional swimming effort. This possibility is also supported by the lower levels of lactate and the identical frequencies of drifts and sprints in R0HP and R1HP (Figure 4.6B). Still, when subjected to R2HP, *L. bocagei* were attracted to the side of the structures near the flume wall (Figure 4.6A), which may correspond to areas of reduced locomotion effort. It is well-established that fish can take advantage of the velocity, turbulence, size and periodicity of vortices (Figure 4.7Figure 4.9) (Liao, 2007, 2004; Smith et al., 2005). This is because as undulatory swimmers, fish are able to take advantage of the interactions between their body and the local hydrodynamic environment (Liao, 2007). In nature, these individual interactions result from the existing physical properties such as flow fluctuations present in geometrically heterogeneous habitats. For habitat mitigation purposes, heterogeneity should be considered as a crucial design parameter, while carefully considering the local hydrodynamic conditions, otherwise it may produce the opposite effect (Smith et al., 2014). Indeed, it is established that fish require a wide range of structures for refuge. Fish have been observed avoiding rapid flow conditions by choosing low flow areas for flow-refuging (Arnekleiv et al., 2004; Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012; Vehanen et al., 2000), hiding in available rocky substrates (Chun et al., 2011), and using lateral refuge (Ribi et al., 2014).

Despite the higher lactate levels observed in R2HP, there was no visible indication that *L. bocagei* were exhausted, as the fish were able to maintain the burst swimming during the total duration of the event. Mean lactate levels found in a fish passage study, where *L. bocagei* was subjected to a 110 $l.s^{-1}$ for 90 minutes, were higher (6.69±1.95 mM) than those registered in this study (5.06±0.49 mM) (Romão et al., 2018). Thus, the results from this study may reflect physiological adjustments for *L. bocagei* to cope with the created hydrodynamic changes. Considering glucose, previous studies have shown increased levels for *L. bocagei* subjected to the most extreme hydrodynamic changes (Costa et al., 2018a,b). In this study, no clear association was found.

The lowest movement behaviour frequency observed in R2BF suggests that this event-configuration is the most beneficial for *L. bocagei*. The lower frequency of drifts observed for individuals and groups suggests that this combination favoured both *L. bocagei* to hold station without significant energy expenditure (Figure 4.6B, Table 4.2). On the other hand, the reduced frequency of structure use may indicate that the hydrodynamic conditions created in the flume area were not adverse enough to induce refuge use (Figure 4.6A, Table 4.2). For all studied configurations under HP, the observed drifts were most commonly associated to a higher frequency of sprints (Figure 4.6B, Table 4.2). This could be motivated by an exploratory behaviour to find suitable flow areas. For example, when brown trout were exposed to fluctuating flows (repeated up- and down-ramping events) the possibility to hide in available

substrate denoted a compensatory behaviour, where after being subjected to repeated stimuli brown trout was able to adjust both physiologically (return of cortisol to pre-stress levels) and behaviourally (hiding in substrate) to avoid negative effects (Flodmark et al., 2002). This compensatory behaviour suggests an adaptive mechanism for juvenile brown trout to re-establish homeostasis (Flodmark et al., 2002; Schreck, 2000). In this study, it was found that the absence of such suitable areas motivated upstream sprinting, by crossing the structures or sprinting upstream to negotiate with the created flow conditions. Sprinting behaviour was more evident for *L. bocagei* during R2HP. These results may indicate that the severity of the flow event together with the presence of R2 may have not provided sufficient suitable areas to recover forcing individual behaviour. Conversely, the hydrodynamic conditions in R0 and R1 seemed to induce drifting (Figure 4.6B, Table 4.2). This could reflect an adaptive behaviour to recover from the effort of sprinting as a means to conserve energy and may explain the lower lactate levels observed for R1HP and R0HP (Figure 4.4A and B).

The differences observed in individual and group behaviour between HP and BF (Figure 4.5A and B) show that the changing flow conditions were not always favourable to maintain schooling. Although fish in groups usually benefit from the increased tail beat frequency of the leading fish (Liao, 2007) the existing flow conditions under HP were not always favourable. Group dynamics was particularly marked under R1BF indicating that the lower discharge and pressure magnitudes favoured group stability. The advantages of schooling behaviour have been demonstrated to reduce the total swimming costs under turbulent flows (Enders et al., 2005) and during reproductive migrations (Standen et al., 2002; Wang and Chanson, 2018).

From the analyses of the physiological and behavioural results together with the artificial lateral line probe measurements, the asymmetry was the most related with behavioural observations. This was particularly evident in the two extremes R2BF and R2HP. Asymmetry, when used as a local flow variable enabled a comparison of the cycles of large-scale vorticity between events, which fish may use to reduce the costs for swimming (Liao, 2007). Considering the different flow event and structure trials, a "favourable asymmetry window" was observed in R2BF for *L. bocagei* (Figure 4.9, Table 4.3). This critical threshold for asymmetry corroborates the third hypothesis, that local hydrodynamic variables exist generating unique movement behaviour patterns. This was particularly evident for R2BF and R2HP (Figure 4.9). The thresholds for the pressure variables observed in R1HP and R0HP (Table 4.3) resulted in similar frequencies of sprints and drifts (Figure 4.6B). These results may denote drifting as a compensatory behaviour to the effort required to sprint. It has been suggested that in rivers affected by hydropeaking fish may change their position by moving backwards and forward between areas with suitable velocity and depth to avoid the negative effects of flow fluctuations (Flodmark et al., 2002).

Each event, HP and BF, combined with R1 or R2, generated unique local flow conditions which were more or less favourable for fish. Considering this, a geometrically heterogeneous configuration of instream structures (i.e. different shapes and sizes) could increase the probability for fish to find suitable refuge areas. As demonstrated, in the absence of suitable flow areas or under hydropeaking conditions, fish benefit from the presence of instream structures for flow-refuging. Nonetheless, complex habitats may also create unfavourable hydraulic conditions for fish (Auer et al., 2017; Goettel et al., 2015). Experimental concepts and engineering design of instream mitigation structures, should therefore take into account the interaction between channel morphology and water level changes (Schmutz et al., 2015).

4.1.6 Conclusions

This study provides experimental evidence that rapidly changing flow events in the presence of instream structures can trigger differentiated physiological and behavioural responses in *L. bocagei*. The LLP results demonstrated that the combination of flow events with instream structures resulted in unique flow conditions, characterized by distinct velocity, turbulence and vorticity distributions in the flume, and particularly near the structure area. As these hydrodynamic features were unique to each combination, and specific behavioural responses were found, critical thresholds of local hydrodynamic variables for *L. bocagei* were defined accordingly (Table 4.3). In summary, under HP conditions lower velocities and higher turbulence and vorticity promote individual structure use, whereas higher velocities and milder turbulence and vorticity were found to promote sprints and drifts. Under BF conditions, the more frequent group behaviour and the lower refuge use, indicate that milder velocities in the structure area and lower mean turbulence and vorticity in the remaining flume area create favourable flow conditions for *L. bocagei*.

Finally, lower discharge magnitudes, and milder flow-ratios (< 8.6), are advantageous for this species and have the potential to reduce the energetic costs associated with rapid flow fluctuations. However, the simultaneous absence of a glucose response and exhaustion signs when *L. bocagei* were subjected to 60 $l.s^{-1}$ encourage further investigation to understand the extent to which flow event-configurations combinations in rapid flow fluctuations trigger physiological responses in *L. bocagei*. Additionally, combined effects which consider the range of environmental factors that fish experience *in situ* , such as thermopeaking, are worthy of further investigation. To benefit from the individual interactions with the velocity, turbulence and vorticity of the hydrodynamic environment, diverse sets of instream structures should be preferred as potential flow-refuging areas for *L. bocagei* subjected to hydropeaking. Further research is encouraged to establish a mechanistic and quantifiable link between the derived pressure

variables and specific fish responses, particularly the ability of fish to demonstrate adaptive mechanisms (e.g. compensation by searching favourable pressure thresholds) to avoid the negative effects of rapid flow fluctuations.

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5

General discussion

“Even the upper end of the river believes in the ocean”.

William Edgar Stafford

5.1 Research overview and discussion

This thesis was divided into two main parts: the first consisted of a systematic review aiming at finding evidence for flow variability as an organism-level stressor. The second consisted of a sequence of experimental trials where the behaviour of *L. bocagei* was assessed according to simulated flow events and diverse velocity refuges.

In chapter 1 the importance of flow regime was discussed, the inevitable deterioration of river ecosystems due to hydroelectricity demand was emphasized, and the responses of fish to flow variability were argued. The crucial role of flow regime for the ecological integrity of riverine ecosystems is undeniable (Lytle and Poff, 2004; Poff et al., 1997). The organization of fish communities along the *river continuum* gradient is determined by the inherent variability of the flow regime components (Poff et al., 1997). However, the mechanistic link between flow variability, either natural or anthropogenic, and a quantifiable fish response remains unknown (Costa et al., 2017). To find evidence for this cause-effect relationship, and to identify potential biomarkers as surrogates of that relationship, the first part of this thesis consisted of a systematic review where a combination of keywords was used as a search string in the Thomson and Reuters *Web of Science* database (Chapter 2). The literature was analysed, temporal and geographic trends were illustrated, and the potential of physiological responses as biomarkers for flow variability was argued. This review firstly showed that flow variability can represent a potential stressor for fluvial fish in conditions beyond their optima. Additionally, the review was the

starting point for the second part of this thesis, contributing for the selection of the physiological responses to be quantified while assessing whether hydropeaking presented a real stressor for *L. bocagei* or not. While answering to the specific questions below, the physiological findings of the second part of the thesis (chapters 3 and 4) will also be integrated in *Part I* (see below) as they corroborate the scientific evidence reported in chapter 2.

The phenomenon of hydropeaking reflects the increasing global demand for a rapid and cost-effective source of energy. The consequent downstream habitat deterioration and flow homogenization will likely continue to affect the organization of downstream fish communities (Young et al., 2011). According to the first scientific article of this thesis (see 2.1), to capture the cause-effect relationship between a flow component change and a quantifiable fish response in natural conditions is challenging. In natural conditions it is possible to get a closer representation of the reality (Woodward et al., 2010). Under experimental flume conditions it is possible to control the factors under examination, isolate confounding variables, and replicate the same experimental conditions (Woodward et al., 2010). In this sense, the choice of an indoor experimental flume was appropriate to study the effects of rapid changes in magnitude, frequency and rate of change, and to associate them to the responses of *L. bocagei*. The results from the experimental part of this thesis present the first evidence of the effects of hydropeaking on the physiology and movement behaviour of *L. bocagei*. The utility of diverse artificial velocity refuges as potential mitigation measures to hydropeaking consequences was also demonstrated for the first time. This research highlights the importance of combining multiple disciplines to achieve more robust results, recognizing the limitations of experimental flume studies to extrapolate the findings to natural conditions. With this multidisciplinary approach it was possible to relate *L. bocagei* responses with the created flow environment. This was characterized using point velocity measurements through ADV technology (chapter 3), and a technology that mimicked, at some extent, the distributed sensing capacity of fish, i.e. the lateral line probe (chapter 4).

This chapter presents an integrative discussion of the major findings of the specific questions that were addressed in chapters 2, 3 and 4. It provides a comprehensive understanding of: (i) flow variability as a stressor for fluvial fish, (ii) hydropeaking as a trigger of physiological responses and movement behaviour shifts, (iii) the utility of velocity refuges as a mitigation measure, and (iv) the relevance of hydrodynamic changes to understand movement behaviour shifts.

Part I: Flow variability as an organism-level stressor for fluvial fish

Does flow variability present an organism-level stressor to fluvial fish? Stress is described as an adaptive mechanism that enables fish to cope with any real or perceived stressor with the objective to maintain the homeostatic state (Barton, 2002) (see 1.4). After analysing the scientific evidence presented in chapter 2, it is safe to infer that any flow change, either natural or anthropogenic, that affects the organism beyond this state, represents a potential organism-level stressor to fluvial fish. The stress response follows a coordinated neuroendocrine pathway which is initiated by stimulus perception (Barton, 2002). The majority of reported physiological changes were related with a transient short-duration stress, i.e. with a transient departure from homeostasis (e.g. Flodmark et al., 2002). Fish were consistently able to return to the physiological conditions referent to the *pre-stress* or resting state (Arnekleiv et al., 2004; Flodmark et al., 2002). Compensation or habituation to flow changes presented potential explanations for absent and inconsistent physiological responses in salmonids, and to their return to *pre-stress* levels (Arnekleiv et al., 2004; Flodmark et al., 2002). Although these mechanisms may represent adaptive fish responses, there is an energetic cost associated with them. In addition, if there is no elevation on the physiological responses that were selected to address the flow disturbance effect, that is not an unequivocal evidence that fish were not stressed (Wendelaar Bonga, 1997).

Habituation, compensation or exhaustion are determined by the severity and duration of the flow stressor (Flodmark et al., 2002). Remarkably, there is no record of an anthropogenic flow disturbance that resulted in the elevation of a physiological response which corresponded to exhaustion (manifested by the inability of the organism to return to the resting state). Thus, the scientific evidence is inconsistent in respect to whether the response becomes maladaptive and results in distress (see 1.4). Nevertheless, the unnatural departure from the resting state associated with artificial flow fluctuations is detrimental as it results in reduced growth (Finch et al., 2015; Korman et al., 2011), increased metabolic costs (Geist et al., 2005), delay in migratory routes (Burnett et al., 2014), among others. These results illustrate that although the adaptive physiological responses to artificial flow disturbances are not maladaptive *per se*, they affect the success of diel and life-cycle events.

Is it possible to use a stress-axis response as a biomarker of flow variability? The answer to this question was not a straightforward “yes” or “no”. The reported baseline cortisol levels representative of unstressed fish are < 10 $ng.ml^{-1}$ (Pankhurst, 2011), or even < 5 $ng.ml^{-1}$ (Pickering and Pottinger, 1989). Regardless of the stressor, these cortisol levels could represent the baseline for an absent stress response. Referring specifically to flow variability, it was rarely considered as an environmental disturbance susceptible of triggering an elevation of the cortisol levels of fish. The major constraints to

use this physiological response as a biomarker for flow variability were related with: the time from flow stimulus perception to blood collection, the effect of external confounding variables, species-specific traits, and intrinsic characteristics of the sampling site. As a consequence, there is no scientific evidence for a baseline cortisol level related with flow variability above which fish will potentially incur in a stress response. Moreover, some physiological responses are more specific to a certain stressor than others (Leatherland, 1985). According to the literature review, the amount of variability and inconsistency were better explained when more than one of the stress-axis responses were used. Thus, to identify valid physiological responses as biomarkers for flow variability is challenging.

This literature review was valuable for the second part of this study, i.e. the experimental part, as it brought novel understanding considering the selection of an adequate response for the hydropeaking problematic. Rapid elevations in cortisol levels are likely to occur within minutes (Pankhurst, 2011). To investigate the rapid changes of specific flow components, longer times of exposure are required. In experimental flume studies cortisol would be adequate if it would be possible to quantify it continuously. In this sense, as secondary level responses, glucose and lactate were identified as the most adequate to quantify in *L. bocagei* subjected to the tested flow events. The reasons to choose glucose were: (i) it generally increases after the cortisol response through the cortisol-mediated gluconeogenesis (Bracewell et al., 2004; Pankhurst, 2011) (see 1.4); thus there is a time latency between cortisol and the glucose response, (ii) it has been broadly used in flow variability studies with reported increments comparatively to *pre-stress* levels (Arnekleiv et al., 2004; Krimmer et al., 2011). Following the scientific recommendation that more than one response should be quantified (Flodmark et al., 2002), the other physiological response selected was lactate. Lactate is a metabolite of muscle activity, and is expected to increase during continued swimming effort (Kieffer, 2000). To obtain such flow conditions, it would be necessary to systematically and continuously test flow variability scenarios until the flow conditions below which fish were able to maintain that state were reached. Three main constraints may explain the absence of that flow threshold: (i) inter- and intra-specific variability (Barton, 2002), (ii) the diverse approaches to study flow variability (e.g. whether the study is conducted in natural or in experimental flume conditions), and (iii) the nonuniversal character of a physiological response (Leatherland, 1985) (some responses are specific to the type of stressors). Given these major constraints, the absence of this universal flow threshold seems reasonable and the effort to find it may be counterproductive.

Is there a universal flow threshold that does not result in a departure from the homeostatic state? The answer to this question was argued in the first research article of this thesis. There is no universal flow threshold representative of that homeostatic state. To obtain such flow conditions, it would be necessary to systematically and continuously test flow variability scenarios until the flow conditions below which fish were able to maintain that state were reached. Three main constraints may explain the absence of that flow threshold: (i) inter- and intra-specific variability (Barton, 2002), (ii) the diverse approaches to study flow variability (e.g. whether the study is conducted in natural or in experimental flume conditions), and (iii) the nonuniversal character of a physiological response (Leatherland, 1985) (some responses are specific to the type of stressors). Given these major constraints, the absence of this universal flow threshold seems reasonable and the effort to find it may be counterproductive.

During the experiments, there was no unequivocal evidence for a flow threshold that did not elicit an increment in either glucose or lactate levels. Contrarily to what would be expected, the physiological responses were not consistently lower under base flow conditions (continuous 7 $l.s^{-1}$). Instead, they fluctuated according to the combination of flow event and structure configuration, and this was more evident for the glucose levels. With the exception of the hydropeaking event with v-shaped structures, the levels of blood lactate did not differ significantly between events and the movement patterns of *L. bocagei* were not indicative that fish were exhausted. Focusing exclusively in the physiological findings, the flow field changes created under 7 and 20 $l.s^{-1}$ in the presence of deflectors, and the predictability of the hydraulic conditions in the absence of instream structures, resulted in the absence of a physiological response in young adults of *L. bocagei*. This is a further step regarding the definition of a flow threshold for the resting state of this species. Moreover, it emphasizes the importance of habitat heterogeneity as a decisive factor for the definition of the most adequate conditions for this species. The concomitant analysis of the movement patterns was decisive when finding the most suitable flow event-structure configuration for *L. bocagei* (but this will be discussed in the next questions - Part II).

Part II: Hydropeaking: Indoor flume experiments

Was there a clear effect of hydropeaking for L. bocagei? The effect of the simulated hydropeaking events was not straightforward, demonstrated by the diverse physiological responses and movement behaviour patterns observed during the progress of the experiments. After initially testing two peak discharges, i.e. 20 and 60 $l.s^{-1}$, it was clear that under the highest discharge in the presence of meandered deflectors, (i) the onset of a glucose response occurred sooner, (ii) the use of deflectors as velocity refuges was contained, and (iii) the sprinting and drifting activity was higher (see 3.1.4 and 3.2.5). Additionally, event duration and peak frequency dictated a sustained glucose response in the 60

$l.s^{-1}$ flow events, and the unpredictability of the two-steps event (i.e. 20 $l.s^{-1}$ followed by 60 $l.s^{-1}$) resulted in elevated glucose levels. From these findings it would be tempting to suggest that there was a clear adverse effect of the hydropeaking events. However, signs of exhaustion were undetected, corroborated by the absence of a lactate response, and fish were able to cope with water velocities > 60 $cm.s^{-1}$ by hiding in the deflectors. Still, the ability of *L. bocagei* to cope with the hydraulic conditions created under 60 $l.s^{-1}$ was lower and, the swimming activity decreased particularly after peak repetition. The unpredictability of the events when more than one of the flow components (e.g. magnitude and frequency) was altered resulted in the most visible responses. Longer peak flows, with higher magnitudes and repeated, resulted in glucose increments, increased swimming effort, and difficulty to hide in the available refuges. Similar findings occurred in the presence of one-sided deflectors. This cumulative effect is recognized in the literature (Harby and Noack, 2013; Young et al., 2011). On the other hand, when a hydropeaking event was tested in the absence of these structures, the lowest physiological responses were obtained. The highly predictable and homogenous flow conditions explained these results. After replacing the deflectors by the instream structures (see 4.1.3), the effects of the same flow event differed significantly between the tested configurations. After this experiment it was clear that the hydraulic conditions created by the combination of the flow event and the tested instream structures were critical for the range of responses showed by *L. bocagei*. This key finding is supported, for example, by studies that were conducted with field data following simulations with hydraulic models (Hauer et al., 2014). Fish have the ability to make use of the surrounding flow conditions to favour rheotactic behaviour (Bak-Coleman et al., 2013; Montgomery et al., 1997; Young et al., 2011) or by taking advantage from vortex structures as strategic areas to save energy associated with swimming activity (Liao et al., 2003). In summary, the effects of hydropeaking can be detrimental but also beneficial (Young et al., 2011). It is the combination of the flow event with habitat heterogeneity that determines it.

According to the responses of L. bocagei, which were the most favourable hydraulic conditions? The physiological responses and the movement behaviour patterns indicate that the most favourable hydraulic conditions were those that created the most predictable flow environment for *L. bocagei*. This conclusion is partially sustained by the generally lower levels of physiological responses and the observed movement behaviour patterns in the hydropeaking events without structures (see 3.2.5 and 4.1.4). Under these conditions, the hydrodynamic changes favoured group drifts. These were considered a compensation mechanism to minimize the effort associated with sprinting (higher tail beat frequency), because there were no available low velocity areas for flow-refuging. Although this mechanism was not addressed in this thesis, studies have demonstrated a high correlation between tail beat frequency and increased costs of swimming (Bainbridge, 1958; McLaughlin and Noakes, 1998). These findings provided

novel insight regarding the possibility of *L. bocagei* to exhibit compensatory mechanisms, even though to find bare habitats in natural conditions is unrealistic. In this sense, the most favourable hydraulic conditions for *L. bocagei* were those where the lower changes in flow components were simulated. These events were: base-flow events in the presence of deflectors and v-shaped structures and in the single-peak events where the lowest peak discharge, i.e. 20 $l.s^{-1}$, was tested. In these events there were no physiological adjustments, the swimming effort was low (demonstrated by the lower frequency of individual sprints and the balanced frequency of individual and group behaviour), and the refuges were easy to access.

In which flow event-structure configuration combination was observed the most ecologically relevant responses in the context of hydropeaking? As it would be expected the overall structure use and swimming activity of *L. bocagei* were more pronounced in the hydropeaking events where a 60 $l.s^{-1}$ discharge was tested. However, the exceptions to this trend are those that provide the most ecologically relevant findings for the hydropeaking problematic. In one hand, the presence of lateral structures (deflectors) created a more heterogeneous flow environment, but *L. bocagei* were still able to use them for flow-refuging. Still, the lower search for deflectors under 60 $l.s^{-1}$ in comparison with the 20 $l.s^{-1}$ events, and the increased overall swimming activity, suggest that the critical hydraulic conditions created in the vicinity of the deflectors reduced the ability to find flow refuge downstream of the deflectors. On the other hand, the combination of flow event with the presence of instream structures altered the distribution of velocity, turbulent fields and pressure fluctuations, generating distinct behavioural responses (see 4.1.4). The movement behaviour of *L. bocagei* was not solely related with the unpredictable flow conditions resulting from the peak discharge (i.e. 60 $l.s^{-1}$). Specifically referring to the instream structures, under the single-step flow event the individual and group use were higher for the solid triangular pyramids. The lower velocities expected inside the v-shaped structures, and that would favour *L. bocagei* for flow-refuging, were masked by the complex flow conditions created by these structures. Noteworthy, the frequency of individual structure use was the highest under base-flow conditions for the solid triangular pyramids and the lowest for the v-shaped structures in the same flow event. Even if the swimming activity was more pronounced under 60 $l.s^{-1}$, the physiological responses were not always indicative of stress or exhaustion.

With the adopted multidisciplinary approach it was possible to demonstrate that the most conclusive responses occurred in the events where more than one flow component (given as magnitude, frequency or duration) changed, and in the presence of the structures that created the most complex flow conditions. These were: (i) the single-step and two-steps events for 60 $l.s^{-1}$ for meandered deflectors

(see 3.1), (ii) the two-steps and the repeated single-step events for one-sided deflectors (see 3.2), and (iii) in the single-step event for v-shaped structures (see 4.1).

Conceptualizing structural mitigation measures to hydropeaking: do lateral or instream structures effectively mitigate the effects of hydropeaking? Before the present research, there was no evidence regarding the effects of hydropeaking on the behaviour of *L. bocagei.* Additionally, the potential of lateral and instream structures as potential mitigation measures had never been addressed. The reasoning behind the selection of lateral structures was: (i) their broad use in restoration actions (Pretty et al., 2003), (ii) velocity preferences of *L. bocagei* (Ferreira et al., 2007a; Oliveira et al., 2002); (iii) velocity refuges proposed for other species (Ribi et al., 2014), (iv) habitat modelling studies based on suitability curves (Boavida et al., 2015), and (v) in studies aiming at designing effective fishways for this species (e.g. Branco et al., 2013a). The instream structures were conceptualized according to: (i) the results from the previous experiments of this thesis, (ii) fishway research for this species (Santos et al., 2014), and (iii) the potential effect of structures in the hydrodynamic conditions (e.g. the formation of eddies, the importance of hydrodynamic changes to smaller scale movement patterns).

Overall, the responses of *L. bocagei* changed according to the hydrodynamic conditions created by the flow event and the presence of structures. In one hand, the movement behaviour results indicated that *L. bocagei* were using the deflectors more frequently than the instream structures, and that under moderate peaks, their use was enhanced. On the other hand, the physiological responses were not so clear: glucose increments occurred in the presence of both deflector configurations, whereas lactate increments occurred only in the presence of v-shaped structures. With these findings this question emerged: does the higher use of deflectors imply that these structures are more favourable for *L. bocagei* under hydropeaking conditions? The insights from this multidisciplinary approach indicate that it was easier for *L. bocagei* to take advantage from the hydraulic conditions created in the presence of deflectors. Still, the swimming activity (frequency of sprints and drifts) was enhanced by the hydrodynamic changes created by the flow event and the presence of instream structures.

In natural conditions, each case is unique and generalizations have to be made with caution. According to the findings of this research, the most decisive factors for the concept of morphological mitigation measures are the hydrodynamic changes resulting from hydropeaking and the choice of the type, number and positioning of structures. For the critical down-ramping stage, the water connectivity between structures and the main channel has to be assured, and the opening angle must avoid clogging and favour group behaviour (see 3.1.5 and 3.2.7). Although a heterogeneous habitat is recommended, more available refuge areas is not always the most adequate solution (Hauer et al., 2014; Tuhtan et al.,

2012). There must be a trade-off between what exists in the river and the effectiveness of adding new structures.

Considering the frequency of structure use, *L. bocagei* were able to use the meandered and lateral deflectors as velocity refuges more effectively than the two triangular shaped structures. Analysing the hydraulic conditions given by the FLOW-3D® models for deflectors and by the LLP for the instream structures, it was evident that rather than just velocity, it was the combination of local scale hydrodynamic features that determined the movement patterns. These explained the difficulty for *L. bocagei* to use the triangular structures as velocity refuges, but also their ability to use local hydrodynamic changes to find other areas for flow refuging (see Liao, 2007), move upstream (favouring rheotactic behaviour) (e.g. Bak-Coleman et al., 2013; Kanter, 2003), or to benefit from the group behaviour (e.g. Liao, 2007).

Did fluid-body interactions provided further insight to a degree that they should be considered to study the effects of rapid flow fluctuations and for the design of mitigation measures to hydropeaking? The results from the artificial lateral line probe provided unique findings on the role of the hydrodynamic conditions as basic triggers of movement patterns of *L. bocagei.* After combining the fish responses with the derived pressure variables, it was possible to define pressure thresholds referring to mean front pressure, mean pressure fluctuations and mean pressure asymmetry (see Table 4.3) that provided the most and the least favourable hydrodynamic conditions for *L. bocagei*. Asymmetry was the pressure variable that was the most related with *L. bocagei* responses. A favourable asymmetry window was observed for the base-flow event with v-shaped structures. On the other hand, the highest pressure asymmetry and the high mean front pressure and pressure fluctuations measured in the single-peak event for the same structures, hindered the fish ability to cope with the hydrodynamic conditions and resulted in a higher swimming effort (lactate adjustments). These were firstly indicated as potential surrogates of local hydrodynamic stimuli according to the fish responses.

As expected, new questions emerged. The results from this experiment are promising considering the role that local-scale hydrodynamic changes have on the swimming performance of fish. Thus, further studies are encouraged. Furthermore, this answer is complementary with that referring to the existence of a flow threshold representative of a resting state. The results reported in 3.1 and 3.2 demonstrated that for lateral structures (i.e. the deflectors), velocity magnitudes ranging from near 0 to 0.41 $m.s^{-1}$ allowed *L. bocagei* to use the available flume area, maximizing this species swimming performance, without any physiological response. The hydraulic characterization given by the FLOW-3D® models was relevant to explain deflector use and swimming activity patterns. However, the information that the

velocity ranges provided was not sufficient to explain the diversity of *L. bocagei* responses. Considering the range of pressure readings by the lateral line system (Kalmijn, 1988), the LLP represents a potential tool to assess the distributed sensing capacity of fish. Thus, in comparison with the ADV, it is expected that the LLP provides a better representation of how specific hydrodynamic changes affect the movement behaviour of fish. This was the first study that used this technology to understand smaller-scale movement behaviour patterns of fish associated with flow variability. Thus, this research represents a step forward to understand the ecological significance of the pressure field around the fish and its movement patterns. These smaller-scale movement patterns have implications between individuals among schools. Using an analogy referring to the hierarchical nature of the river system, the dynamics of riverine microhabitats will be reflected at the broader basin scale; thus, these individual movement patterns will likely affect school dynamics, populations, communities and ultimately the ecosystem.

5.2 Research limitations

The progress of a study based on hypothesis formulation and testing is always shadowed by continuously emerging challenges. Some lead to new hypothesis and consequently to scientific progress, while others do not. For this research, the main aim was to study the effects of rapid flow fluctuations associated with hydropeaking. The main challenge was to simulate extreme flow conditions due to the construction characteristics of the indoor flume. The maximum discharge of this facility was limited to 60 $l.s^{-1}$. It was possible to partially overcome this limitation by including two parallel deflectors in the upstream area of the flume in the meandered deflectors' configuration to reduce the cross-section. With this solution it was possible to achieve higher velocity ranges during the hydropeaking events (see 3.1.3). It was visible that the deflectors were used for flow-refuging, however their presence created a more complex flow environment. Under 60 $l.s^{-1}$ it was more difficult for *L. bocagei* to successfully find them. In this sense, although there was this construction limitation, the effects of the rapid flow fluctuations was evident. However, after this experiment the effect of smaller hydrodynamic changes on the movement patterns of *L. bocagei* stood out. These findings resulted in new hypothesis formulation to understand those behaviour patterns according to the created hydraulic conditions (see 4.1).

Experimental flume research has been encouraged to understand the effects of rapid flow fluctuations in downstream fish communities (Harby and Noack, 2013; Young et al., 2011). In laboratory conditions fish behaviour is exhibited similarly to those in natural conditions (Mittelbach et al., 2014). Thus, the experimental flume approach is reliable to effectively study behaviour patterns. However, to generalize

behaviour patterns according to flow variability and extrapolate it to natural conditions is discouraged. Nevertheless, the novel recommendations herein proposed were only achievable due to the possibility to examine the movement behaviour patterns of *L. bocagei* in the experimental flume. Ideally, experimental flume studies should be complemented with *in situ* studies to include the control and replicability conferred by experimental flume conditions, and the realism inherent to natural conditions.

Another study limitation was related with the replicability of the methods and findings. One of the biggest problems of ecological studies, commonly discussed among scientists, is the difficulty to replicate these experiments and their findings. These studies are usually constrained by the number of organisms that it is possible to obtain from the natural conditions, reducing the statistical robustness of the findings. In this thesis, this limitation was partly outweighed by using a diverse set of behaviour quantification tools (physiology and movement behaviour metrics), complemented with a detailed characterization of the hydraulic conditions, and the use of statistical tools that are adequate for small-sized samples. This approach strengthened the findings of this research.

The target species of this thesis was *L. bocagei.* Although there are few studies investigating the effects of hydropeaking on cyprinids, the study of a single species makes it difficult to extrapolate to communities or the river ecosystem. Although the single-species limitation is recognized, the scientific value of this thesis for the hydropeaking problematic remains evident.

5.3 Practical applicability of the research

The natural flow regime framework postulates that the inherent variability of its flow components has evolved ecological and evolutionary traits in fish (Allan and Castillo, 2007; Bunn and Arthington, 2002; Poff et al., 1997). It would be logical to generalize that hydropeaking consequences are detrimental for downstream fish communities. This thesis demonstrated that hydropeaking generates diverse physiological responses and movement behaviour patterns. In a long-term, the responses could be detrimental (for example due to the increased swimming effort associated with continuous high peaks, or unpredictable flow conditions), but may also favour adaptive responses.

The practical applicability of this research can be ramified into: (i) recommendations for the operational scheme, and (ii) implementation of morphological mitigation measures. The reason to begin with the operational recommendations arises from the unexpected results regarding the two-steps events (see 3.1.4 and 3.2.5). The elevated glucose levels, the inability to find the deflectors particularly during the transition between discharges, and the increased swimming activity in the second step demonstrated

that this type of flow event is not favourable for *L. bocagei*. The explanation for these results refers to the difficulty of *L. bocagei* to cope with the unpredictable flow conditions. This finding is appealing for hydropower production managers, as the economic benefit of a two-steps event, would be lower than a continuous high peak single-step event, for example. Nevertheless, between two high peaks it is recommended that the duration of the base-flow conditions is extended to enable fish to recover from the effort required to cope with the continuous high peak.

Regarding the implementation of morphological mitigation measures, the results were not straightforward for the successful effect of lateral or instream structures as velocity refuges for *L. bocagei*. Once again, the diverse fish responses explain the inability to make such generalizations. However, the refined scale of this study, considering the movement behaviour patterns according with the hydrodynamic stimuli, together with the organism-level responses were decisive for those conclusions. The possibility to establish this causality, otherwise difficult to figure out in riverine conditions, is fundamental to prevent the implementation of unnecessary, and most of the time, costly mitigation measures. The combination of the flow event with the presence of a structure was the most determinant factor for the onset of a fish response. In this sense, this study proposes guidelines to implement lateral deflectors in natural conditions (see 3), and emphasizes the importance of habitat heterogeneity, reinforcing that “more” is not a synonym of “adequate” (see 4).

5.4 Final remarks

Part I: Flow variability as an organism-level stressor for fluvial fish

Flow variability has the potential to trigger a transient departure from homeostasis that corresponds to an adaptive response. Following this transient short-duration stress, habituation or compensation mechanisms may occur, which correspond to the adaptive response aiming at re-establishing homeostasis. From the analysed literature, there was no evidence of such a severe flow disturbance that resulted in exhaustion where fish were no longer able to regain homeostasis. Given the vast possibilities of fish responses, to find a suitable primary or secondary level response to be used as a biomarker for flow variability is challenging and may be unproductive. The main reasons contributing for this knowledge gap are related with uncertainties respecting: the flow disturbance under scrutiny, the affected organism, and the selection of the physiological response. The vast amount of flow fluctuations that are possible to study depend on the type of experiment (is the study conducted in natural or controlled conditions?), the timespan between the onset of the flow stimulus and sample collection

(should it be extended or reduced?), and the potential effect of external variables (e.g. water chemistry, river morphology, time of day). Moreover, the choice of the target fish species also adds uncertainty. Not only there is a great amount of variability between species, but between individuals of the same species. Finally, the physiological responses to a disturbance are not universal; they change in relation to the stressor but also with the fish species.

The absence of scientific evidence for a flow threshold that corresponds to the resting state (homeostasis) or from which deleterious effects occur (distress) is one of the major gaps considering flow variability as a potential organism level stressor. Although there is an accepted cortisol level corresponding to the resting state in fish (< 5 or 10 $ng.ml^{-1}$) there is no evidence for a flow threshold that corresponds to that state.

Part II: Hydropeaking: Indoor flume experiments

The novel and multidisciplinary approach used in this research contributed greatly to understand smaller scale behavioural patterns of *L. bocagei* and to link them with the hydrodynamic changes created by the flow events and the refuge configurations. The initial objectives that were proposed for this thesis were progressively addressed. A great part of them was achieved. However, as new questions emerged, the new advances and the novel perspectives widened the scope of this research.

This research has firstly demonstrated the impacts of the rapid and artificial flow fluctuations associated with hydropower production for *L. bocagei*. According to the simultaneous analysis of physiological responses, movement behaviour patterns, and changes in flow components, it was possible to provide guidelines for the design and implementation of structures for flow-refuging, and recommendations for operational measures in the hydropower plant. In addition, for the flow events and refuge configurations tested it was possible to define suitable flow thresholds that represented the resting state of *L. bocagei*, which were in accordance with velocity preferences for this species (Martínez-Capel and García de Jalón, 1999; Mateus et al., 2008). Finally, grounded in the functional principles of the fish lateral line, by using a novel technology it was possible to firstly relate local hydrodynamic changes, given by pressure variables, with specific movement behaviour patterns and physiological responses of *L. bocagei*. By integrating those results, it was possible to define pressure thresholds that represented the least and the most favourable flow event-instream structure combinations for *L. bocagei* (Table 4.3).

Although it was possible to provide design guidelines for lateral deflectors and recommendations related with habitat heterogeneity and operational measures, one must acknowledge that each river

affected by hydropeaking is unique and those guidelines and recommendations must be adapted to each case. In spite of the limitations of indoor flume experiments, this study demonstrated that examining fish responses (physiological and movement behaviour) in combination with hydraulic models and fluid-body interactions, it is possible to get a comprehensive understanding of the fluid-fish relationship. This possibility would be inhibited if using a single approach. The refined scale of this study demonstrated the potential negative aspects of the hydropeaking problematic, but also the opportunities that can emerge from them. These last refer specifically to the advantages of, for example, stable high flows to elicit positive rheotaxis, facilitating the access to flow-refuging areas, assuring the consistency of group drifts, or the possibility to take advantage of lower hydrodynamic stimuli such as vortices, to find flow refuges to recover from the swimming effort. Moreover, what appeared to be a favourable flow event for *L. bocagei*, referring specifically to the two-steps event, and unprofitable for water managers, revealed to be the other way around. Acknowledging the growing increase for a renewable and cost-effective source of energy, the demand for hydroelectricity will continue to grow. According to the findings of this research, to avoid the implementation of costly and ineffective mitigation measures, the study of individual responses and inter-individual interactions in indoor experimental flumes studies is encouraged.

5.5 Future research

The present thesis provided substantial contribute regarding the hydropeaking problematic. According to the findings of this thesis, the relevance of the hydropeaking problematic, and the global demand for a renewable and cost-effective energy source, the further research recommendations are:

1) Given the particular role of event predictability for *L. bocagei*, the study of alternative up- and down-ramping rates is strongly advised. It is predicted that the slower onset of the up-ramping stage will enable the fish to gradually adapt not only to the severity of the peak flow, but to the change in the water level. In the same way, slower down-ramping rates have the potential to reduce the stranding probability as the gradual flow decrease may function as a cue for fish to escape from potential isolated pools. Particular focus should be addressed to the critical down-ramping stage as this is usually associated with reduced chances of survival and high mortality rates.

2) Habituation and compensation mechanisms have been pointed out as potential adaptive responses to rapid flow fluctuations. In this research, it was verified that long duration peaks with repetition, resulted in physiological adjustments but also in a reduction of the swimming activity during the repeated peak. A new question has emerged with this finding: was the swimming effort higher in the

second peak, resulting in a reduction of sprinting, drifting and inability to find the velocity refuges, or were *L. bocagei* becoming adapted to the peak? To understand whether *L. bocagei* were effectively adapting to the stable high peak, testing more frequent peaks is recommended. If young adults of *L. bocagei* may effectively adapt to peak repetition, stable high peaks alternated with longer duration base-flows, may be more beneficial for *L. bocagei* than, for example, a two-steps event.

3) It is known that the water velocity preferences of this species vary during its ontogeny. For this study the selected subjects were young adults of *L. bocagei*. To avoid biased assumptions that were exclusively grounded in findings for this life-stage, it is necessary to further the investigation to specific bottlenecks, such as the critical recruitment stage, or during reproductive migrations.

4) Rheophily, foraging or reproductive guilds are species specific. One aspect that these have in common is an inherent flow preference. *L. bocagei* is a potamodromous bottom species. Future research should be directed to other species with different habitat guilds. This would bring novel insight of the potential effects of hydropeaking for the structure and organization of fish communities in rivers affected by hydropeaking.

5) With this investigation the identification of a flow threshold that represented the resting state of *L. bocagei* was not straightforward, because there was not enough evidence that demonstrated that a specific flow change resulted in an actual stress response. Fish species have evolved adaptive mechanisms to cope with natural and also to artificial extremes. However, under hydropeaking conditions the frequency of peaks is significantly higher than in a free-flowing river. The associated energy-cost to cope with those rapid and artificial changes is likely proportional. In this sense, although fish are able to adapt to novel conditions, the consequent effort has considerable drawbacks. It will certainly reduce the energy available for diel activities and to successfully complete life-cycle events. If a biomarker for flow variability is established, it will be possible to infer whether or not the flow fluctuations in natural conditions will represent a potential stressor for this species and to act accordingly.

6) With this study it was possible to relate local hydrodynamic changes with specific movement patterns and physiological responses. These findings represent the first step of a novel perspective to study behavioural patterns related with rapid flow fluctuations. Thus, it is still premature to make generalizations. To strengthen the findings regarding fluid-body interactions according to rapid flow fluctuations and habitat heterogeneity it is necessary to establish a quantitative relationship. This quantitative analysis would allow to predict the magnitude of specific movement patterns in accordance

with local hydrodynamic changes and to interpret them in terms of, for example, swimming effort under a rapid flow fluctuations' context.

7) The stability of a fish school is known to reduce the swimming effort of each individual. This study demonstrated that under hydropeaking conditions the individual behaviour is predominant and that, with uncertain extent, the group behaviour is reduced. As *L. bocagei* is a schooling species, it makes sense that the swimming effort to maintain the stability of a school under rapid flow fluctuations will likely increase. In this sense, given the ecological relevance of this behaviour and the findings considering the relevance of local hydrodynamic changes for individual and group behaviour differences, further research effort should be directed to this topic. During the experimental campaigns it was possible to identify specific movement behaviour sequences related with sprinting and drifting (herein termed as sprint-recovery cycles) that were identified, but not quantified. These sequences suggested a compensatory mechanism for *L. bocagei* to rest, and were particularly evident in the experiments without refuges. The predictability of the flow conditions appeared to favour those sequences. The swimming effort associated with hydropeaking conditions, indicates that this and other potential behaviour sequences should be further studied. Understanding these patterns would provide further knowledge for the design of effective habitat enhancement structures that would favour this behaviour under highly fluctuating flow environments.

8) Although it was possible to define guidelines for the design of deflectors and to propose alternative operational measures, it was not possible to upscale them to the river system. This was one major limitation of this investigation, even if it was not included in the objectives of this research. In this sense, it is strongly recommended that future research combines experiments conducted in flumes and in natural conditions.

6

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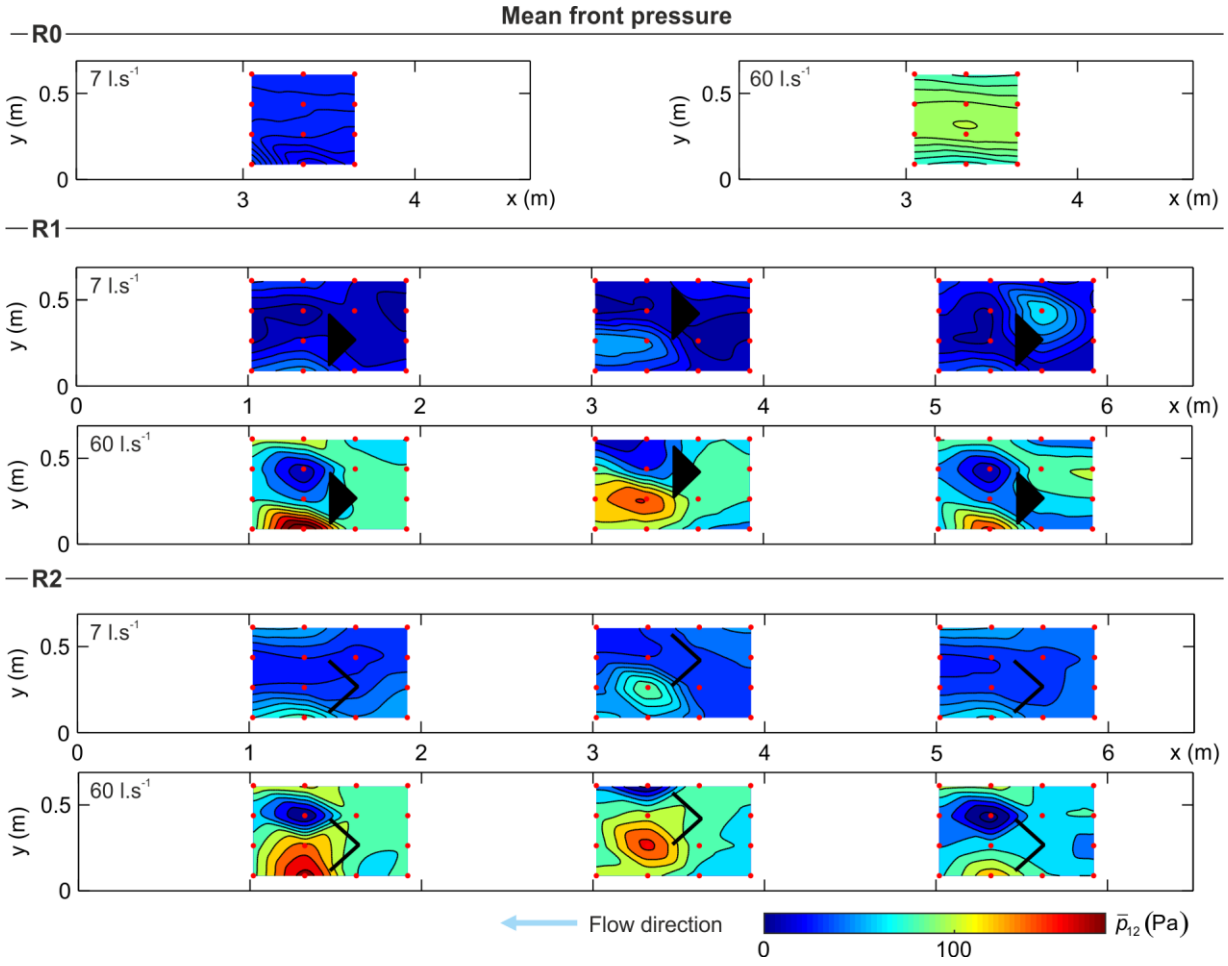
Appendix

Supplement 1 List of references analysed for the review paper (chapter 2), with details for the type of response quantified, Primary (I), Secondary (II) and/or Tertiary (III), hydrological regime and experimental conditions.

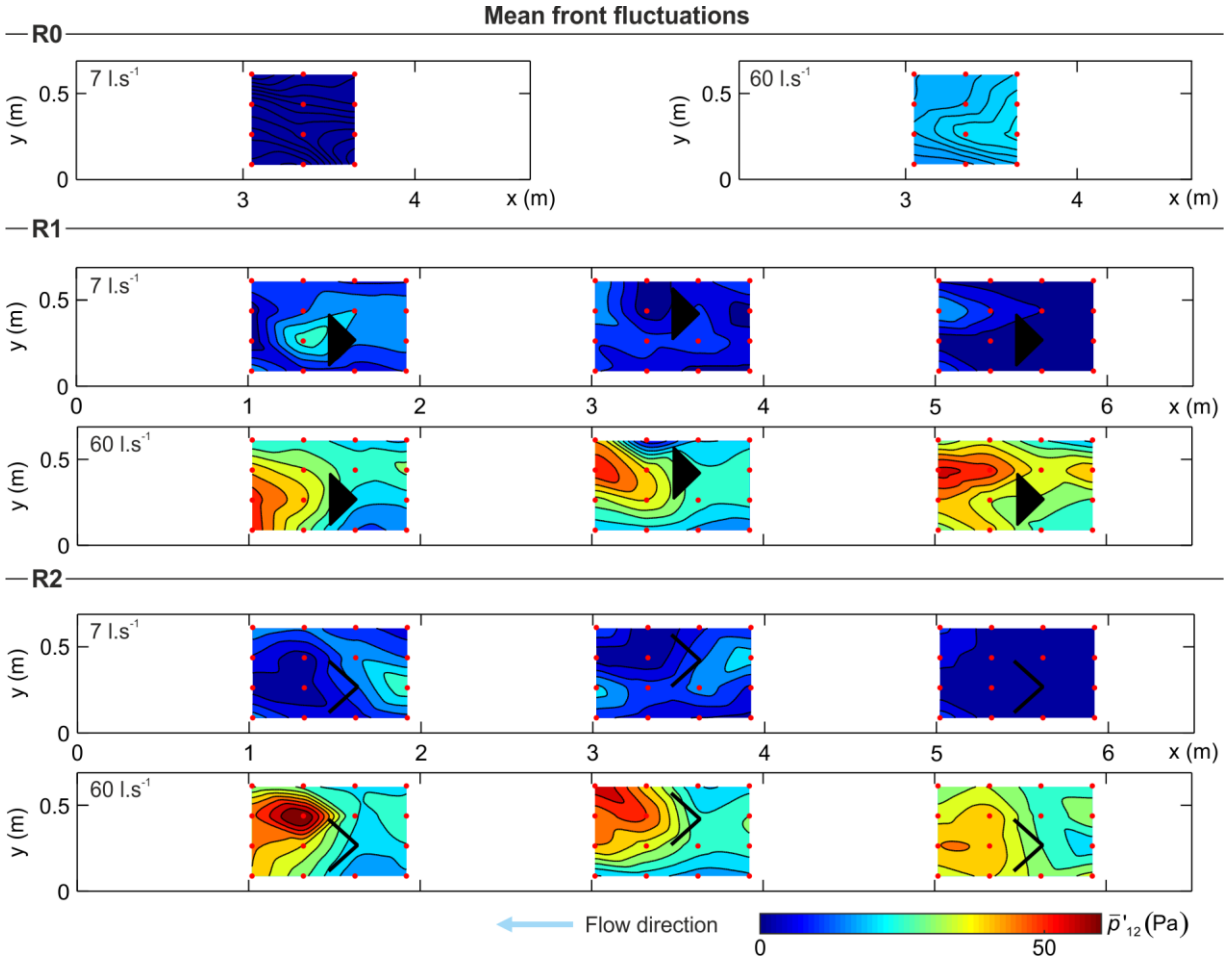
Author	Quantified responses to flow variability	Studied hydrological regime	Experimental conditions
Aniagu et al. 2006	II;III	Modified	Experimental flume
Arnekleiv et al. 2004	I;II	Modified	Artificial stream
Balcombe et al. 2012	III	Natural	River
Burnett et al. 2014	II;III	Modified	River
Burrows 1969	I;II	Manipulated	Tank
Cai et al. 2014	II;III	Modified	Respirometer
Cai et al. 2015	II;III	Modified	Respirometer
Casas-Mullet et al. 2015	III	Modified	River
Cocherell et al. 2011a	II;III	Modified	River/Tank
Cocherell et al. 2011b	I;II;III	Modified	Experimental fishway
Enders et al. 2003	II	Natural	Respirometer
Enders et al. 2005	II	Natural	Respirometer
Farlinger & Beamish 1978	II	Manipulated	Tank
Finch et al. 2015	III	Modified	River
Fisk et al. 2013	III	Modified	Tank
Flodmark et al. 2002	III	Modified	Artificial stream
Flodmark et al. 2004	I;II	Modified	Tank
Flodmark et al. 2006	III	Modified	Artificial stream
Fu et al. 2013	II;III	Modified	Respirometer
Fu et al. 2014	II;III	Manipulated	Respirometer
Fu et al. 2015	II;III	Manipulated	Respirometer
Geist et al. 2005	II;III	Modified	River/Respirometer
Gregory & Wood 1998	I;II;III	Manipulated	Respirometer
Hackenberger et al. 2015	II	Natural	River
Harvey et al. 2006	III	Natural	Artificial stream
Hasler et al. 2012	II	Modified	River
Hasler et al. 2014	II;III	Modified	River
Hockely et al. 2014	III	Natural	Experimental flume
Jensen & Jonhsen 1999	III	Natural	River
Kemp et al. 2006	II;III	Natural	Experimental flume
Korman & Campana 2009	III	Modified	River
Korman et al. 2011	III	Modified	River
Krimmer et al. 2011	I;II;III	Modified	Artificial stream
Maceda-Veiga et al. 2009	III	Natural	River
Milligan etal. 2000	I;II	Manipulated	Tank
Nadeau et al. 2010	I;II;III	Natural	Experimental flume
Nislow et al. 2004	III	Natural	River

Patterson et al. 2004	I;II;III	Manipulated	Tank
Pon et al. 2009	I;II	Modified	River
Pottinger et al. 2011	I;II	Natural	River
Saltveit et al. 2001	III	Modified	River
Sinclair et al. 2014	III	Manipulated	Tank
Sloman et al. 2001	I;II;III	Natural	Experimental flume
Sloman et al. 2002	I;II;III	Natural	Tank
Sneddon et al. 2006	III	Natural	Tank
Sunardi & Manatunge 2005	III	Natural	Experimental flume
Sunardi et al. 2007	II;III	Natural	Tank
Suski et al. 2007	I;II	Manipulated	Tank
Taylor et al. 2012	I;II	Modified	River
Taylor et al. 2013	II	Modified	River
Taylor et al. 2014	II	Modified	River
Teichert et al. 2010	III	Natural	Artificial stream
Woodward & Smith 1985	I;II	Manipulated	Tank
Young & Cech 1993a	I;II	Manipulated	Tank
Young & Cech 1993b	III	Manipulated	Tank
Young & Cech 1994a	I;II	Manipulated	Tank
Young & Cech 1994b	III	Manipulated	Tank
Young et al. 2010	I;III	Modified	Experimental flume

Supplement 2 Contour representation of mean front pressure $\bar{p}_{12}$.



Supplement 3 Contour representation of mean front fluctuations $\overline{p}'_{12}$.



Supplement 4 Contour representation of mean pressure asymmetry $\Delta\bar{p}'_{1-6}$.